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Aniline in finger paints: Health impairments for children shouldn't be expected according to current understanding

Aniline is an important raw material in the chemical industry. It serves as a starting material for the production of various plastics, adhesives and dyes, which in turn are used in the production of consumer products, including toys. Aniline can therefore be present in products as a manufacturing impurity or as an (unremoved) residue after production. It can also occur as a cleavage product of azo dyes which were used to dye the product.

In the EU, toys are subject to safety requirements. The content limit for free aniline in finger paints is 10 milligrams per kilogram (mg/kg). A query to the state investigation offices revealed that such high concentrations have not yet been detected in their controls.

Aniline can harm the nervous system as well as red blood cells. It also has a sensitizing potential, which can result in an allergic skin reaction (contact dermatitis). Rats given high doses of aniline developed tumours of the spleen – thus, a carcinogenic property is assumed for aniline. It is still unclear whether the carcinogenic effects in the (male) rat are based on a threshold mechanism and how or whether these findings are transferable to humans. The observed effects reflect the hazard potential that aniline may pose. The risk of adverse effects from a substance depends on the amount humans are exposed to as well as on the exposure duration.

In the following, the German Federal Institute for Risk Assessment (BfR) has assessed the health risk of aniline in finger paints. The BfR has made the very conservative assumption that a child plays with finger paint regularly from the age of one to fourteen and that the paint always contains the maximum permissible concentration of 10 mg aniline/kg of finger paint. In addition to the intended skin contact, possible intake *via* the mouth was also taken into account for children under the age of 36 months. The margin-of-safety approach was used to assess the non-carcinogenic effects, while the additional lifetime cancer risk was determined with regard to possible carcinogenic effects.

The assessment of the non-carcinogenic effects resulted in a sufficiently large margin of safety. The additional lifetime cancer risk is in the range of 1:1,000,000 which was considered as being just about acceptable by the Scientific Committee on Health and Environmental Risks (SCHER). Since in reality the aniline concentration in finger paints is

significantly lower than assumed here, the probability of occurrence of health effects is very low.

BfR Risk Profile | Opinion [number/year]

Aniline in finger paints

A Affected persons	Children				
B Probability of health impairments from regular contact with aniline through the use of finger paint	Very low	Low	Middle	High	Very high
C Severity of health impairments from regular contact with aniline through the use of finger paint	No impairment	Mild impairment [reversible/irreversible]	Moderate impairment [reversible/irreversible]	Severe impairment [reversible/irreversible]	
D Validity of available data	High: The most important data are available and are internally consistent	Medium: Some important data are missing or inconsistent	Low: A large volume of important data is missing or inconsistent		
E Controllability by consumers	Control not necessary	Controllable with precautionary measures	Controllable by avoidance	Not controllable	

Fields with a dark grey background indicate the properties of the risk assessed in this Opinion
(for more details, see the text of Opinion number [number/year] from the BfR dated [day/month/year]).

Explanation
The risk profile is intended to visualise the risk outlined in the BfR Opinion. The profile is not intended to be used to compare risks. The risk profile should only be read in conjunction with the corresponding Opinion.

1 Subject of the assessment

In the following, the German Federal Institute for Risk Assessment (BfR) has assessed aniline in finger paints. Aniline is used as a raw material for dyes and plastics, for example, which are used in the manufacture of consumer products, including toys. It may therefore be present in products as a manufacturing impurity. In addition to its occurrence in free form, aniline can also be formed through reductive cleavage of certain azo dyes with which the material had been dyed.

According to Regulation (EC) No. 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation), aniline (CAS No. 62-53-3, EC No. 200-539-3) is harmonised classified as a carcinogenic (capable of causing cancer) and mutagenic (capable of inducing changes in DNA) substance of category 2 (Carc. 2, Muta. 2), harmful to organs through prolonged or repeated exposure (STOT RE 1), and skin sensitising (Skin Sens. 1).

Directive (EU) 2021/903 sets a concentration limit for free aniline of 10 mg/kg in finger paints and a limit for aniline released by reductive cleavage of 30 mg/kg in finger paints and in toy material made of textile and leather in Appendix C to Annex II of the European Toy Safety Directive 2009/48/EG (EU Toy Safety Directive). These limit values apply to toys that are intended for children under the age of 36 months or that are intended to be placed in the mouth; they have been applicable since 5 December 2022. According to the EU Toy Safety Directive, a 1,000 to 2,000 times higher limit value of 1% (10,000 mg/kg) currently applies to toys in other categories or to toys that are made from materials other than those mentioned above. This value corresponds to the generic concentration limit in the CLP Regulation for the labelling of mixtures which have a harmonised classification as Carc. 2 or Muta. 2.

2 Results

Regarding the potential of aniline to damage erythrocytes and the central nervous system, no health effects are expected for children of different ages for the maximum permissible aniline content of 10 mg/kg finger paint following simultaneous oral and dermal exposure. Likewise, with regard to a possible carcinogenic effect, even assuming a mode of action without a threshold value and in conjunction with a very conservative exposure assessment, the additional lifetime cancer risk (lifetime excess cancer risk, LCR) would be in the range of 1:1,000,000 (or 1×10^{-6}), which was considered as (just about) acceptable by the Scientific Committee on Health and Environmental Risks in 2010 (SCHER, 2010).

3 Rationale

3.1 Risk assessment

3.1.1 Hazard identification

Aniline (CAS No. 62-53-3) is a clear, colourless to slightly yellowish, oily liquid with a sweet to fishy odour. It has a molecular weight of 93 g/mol, an *n*-octanol-water partition coefficient K_{OW} of 7.94 (log K_{OW} = 0.9) and a water solubility of 36 grams per litre (g/l) (Wellner et al., 2008). Chemically, it is a benzene ring with an amino group ($–NH_2$), making it the simplest primary aromatic amine.

Aniline is an important raw material in the chemical industry. It is used as a raw material for, e.g., the production of various polyurethane plastics, adhesives, rubber additives and dyes, which are used in the manufacture of consumer products, including toys. Therefore, aniline can be present in products as a manufacturing impurity or as an (unremoved) production-related residue. In addition to its occurrence in free form, aniline can also be formed through reductive cleavage of certain azo dyes with which the material had been dyed.

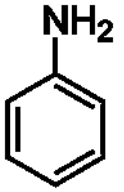

Figure 1: Structural formula of aniline

Current regulations for aniline in toys

According to Article 10 Paragraph 2 of the EU Toy Safety Directive, “Toys shall not jeopardise the safety or health of users or third parties when they are used as intended or in a foreseeable way, bearing in mind the behaviour of children”.

In December 2022, aniline was included in Appendix C to Annex II of the EU Toy Safety Directive with a limit value of 10 mg/kg for free aniline in finger paints, and of 30 mg/kg for aniline released by reductive cleavage of azo dyes in finger paints and in toy material made of textile and leather for toys that are intended for children under the age of 36 months or that are intended to be put in the mouth. For toys of other categories or toys made from materials other than those mentioned above, the EU Toy Safety Directive states that free aniline, which according to the CLP Regulation is classified as carcinogenic and mutagenic category 2 (Carc. 2 and Muta. 2), may theoretically be contained in toys up to a concentration of 1% or 10,000 mg/kg, provided that they do not jeopardise the safety or health of users or third parties when they are used as intended or in a foreseeable way (see Article 10 Paragraph 2 of the EU Toy Safety Directive). This value corresponds to the generic concentration limit in the CLP Regulation for the classification and labelling of aniline in mixtures.

In addition, the European standards of the EN 71 series specify the general safety requirement for toys by defining chemical requirements for certain toys. In addition, analytical methods for verifying the compliance with the limit values are described in the standards. Harmonized standards such as EN 71-7 have been published in the European Official Journal (EU, 2019) and are therefore a legally binding basis for the presumption of conformity, i.e. for compliance with the relevant essential requirements of the EU Toy Safety Directive (EU, 2009). For example, toy manufacturers can refer to the application of relevant harmonised standards in their declaration of conformity in accordance with the EU Toy Safety Directive. By affixing the CE marking (‘Conformité Européenne’, French for ‘European Conformity’) to a product, a manufacturer declares that the product meets the requirements of the EU Toy Safety Directive.

3.1.2 Hazard characterisation

Toxicokinetics

Aniline can be absorbed into the body either orally, dermally or by inhalation. After oral administration of radioactive (^{14}C)-labelled aniline, up to approx. 89% (after 24 h) or 96% (after 48 h) of radioactivity was excreted in the urine in rats; in the mouse it was approx. 72% after 24 h (McCarthy et al., 1985; Bus & Popp, 1987). In a human study with four volunteers, a total urinary excretion of the unconjugated parent substance and three of its metabolites of up to 72.1% was found 48 h after a single oral administration of isotope-labelled aniline-d_5 (ring-deuterated) (Modick et al., 2016). However, since not all relevant

aniline metabolites were analysed, the actual renal elimination and thus also the (preceding) gastrointestinal absorption is probably above the measured 72.1%. Therefore, in the present Opinion, an oral bioavailability of 100% was assumed as the *worst-case* scenario for the calculation of the systemic exposure dose after swallowing toy material containing aniline.

Ex-vivo penetration experiments with excised, non-viable human skin using the Franz diffusion cell showed a penetration rate of 20–38% of the applied quantity, determined as the total amount of aniline recovered in the receptor fluid over 24 h (Wellner et al., 2008). For this purpose, aniline was applied in concentrations of 0.03 g/l in 95:5 (v/v) phosphate buffer/ethanol and of 0.3 g/l, 3 g/l and 30 g/l in 0.9% NaCl solution and as a pure substance (density of the liquid: 1,020 g/l) under *infinite*-dose conditions (500 µl per cm^2). The receptor fluid was collected at several time points between 1 h and a maximum of 24 h after application, and the aniline concentration was determined by gas chromatography/mass spectrometry (GC/MS). The authors did not determine the amount remaining in the skin (dermis and epidermis without *stratum corneum*). However, this information would be necessary to calculate absorption according to the *Notes of Guidance* of the Scientific Committee on Consumer Safety (SCCS) (2021) for the testing of cosmetic ingredients and their safety evaluation. Wellner et al. assumed that "a relevant amount" of aniline remained in the skin.

The data obtained showed an almost linear relationship between the maximum flux rate and the applied aniline concentration. The flux rate indicates the amount of substance per skin surface area and time unit that penetrates the skin and passes into the receptor fluid. Re-analysis of the relationship between the flux rate and aniline concentrations using linear regression through the origin resulted in a permeability coefficient (K_p) of 0.0073 cm/h as the slope parameter for concentrations of up to 30 g/l (= 30,000 mg/kg).

In an *in-vivo* study on ten healthy volunteers, the dermal absorption of aniline was determined based on the urinary excretion of the aniline metabolite 4-aminophenol over a period of 24 h (Baranowska-Dutkeiwicz, 1982). For this purpose, one hand (347 to 459 cm^2 skin area) of each participant was immersed in a 1% and 2% aqueous aniline solution at a temperature of 20 ± 1 °C for 30 or 60 min. The exposed skin area was then cleaned with soap and water. 4-aminophenol was measured in urine over 24 h by using the indophenol method according to Dutkiewicz (1974; cited from Baranowska-Dutkeiwicz, 1982), for which a coefficient of variation of 9.5% was reported. The amount of aniline absorbed through the skin (*x*, mg) was then calculated using the 4-aminophenol excretion rate (*y*, mg/h) determined over a period of 6–8 h after the start of exposure using the following formula from Piotrowski (1957):

$$y = \frac{0{,}14 \times x^{1{,}44}}{8{,}5 + x^{0{,}44}}$$

Piotrowski gives the accuracy of this dose reconstruction as ±35%. Thus, in the *in vivo* study from Baranowska-Dutkeiwicz (1982), the amount of 4-aminophenol excreted in the urine in 24 h reflects 13–55 mol% of the amount of aniline ingested; it is therefore subject to quite high variability. According to Piotrowski (1957), the higher the amount of aniline ingested, the higher the rate of conversion to 4-aminophenol. Interindividual differences probably also exist in the level of conversion to this metabolite. The results of the *in vivo* study from Baranowska-Dutkeiwicz (1982) are summarised in Table 1. The dermal flux rate (µg/cm^2/h)

was calculated by dividing the absorbed amount of aniline by the exposed skin area and the exposure time.

Table 1: Number of trials, exposed hand area, aniline concentration, exposure time and resulting mean absorption rates (in µg aniline per cm^2 and h) of the *in vivo* skin penetration study of Baranowska-Dutkeiwicz (1982).

Number of trials	Exposed hand area (cm^2)	Aniline concentration (%)	Exposure time (min)	Mean flux rate (µg/cm^2/h)
4	347 – 416	1	30	320
4	350 – 436	1	60	200
4	350 – 459	2	30	1,220
2	347 – 370	2	60	820

The results showed mean flux rates between 200 and 1,220 µg/cm^2/h. It was shown for both exposure periods that doubling the aniline concentration from 1% to 2% led to a quadrupling of the flux rate (from 320 to 1,220 µg/cm^2/h after 30 min and from 200 to 820 µg/cm^2/h after 60 min). However, doubling the exposure time (from 30 to 60 min) led to a reduction in the flux rate for both aniline concentrations (Table 1).

The data from Baranowska-Dutkeiwicz (1982) show that uptake does not occur at a constant rate, but happens very quickly at first and then slows down over time. An indication of this is the observation that the amount absorbed after 60 min of exposure is less than twice that after 30 min of exposure. The initially very rapid uptake could be favoured by the strong concentration gradient between the aqueous solution and the skin at the beginning of the experiment.

Compared to the *in vitro* study from Wellner et al. (2008), the *in vivo* study from Baranowska-Dutkeiwicz (1982) resulted in comparatively higher flux rates. In the *in vitro* study, a maximum flux rate of 218 µg/cm^2/h was obtained for a 3% aqueous aniline solution, while the *in vivo* study for the 1% aqueous aniline solution already resulted in an approximately equally high mean flux rate of 200 µg/cm^2/h. One reason for the discrepancy between the *in vitro* and *in vivo* experiments could be the different methodology, i.e. in the experimental setup as well as in the calculation of the flux rates.

The data from the *in vivo* study from Baranowska-Dutkeiwicz (1982) are used to estimate the uptake of aniline from finger paints through the skin, since the experimental setup is closer to the exposure scenario and the flux rates from the *in vitro* study from Wellner et al. (2008) presumably represent an underestimation of the actual uptake through the skin.

Distribution, metabolism, elimination

Orally ingested aniline is quickly absorbed, distributed, metabolised and finally excreted.

After single oral administration of ^{14}C-labelled aniline hydrochloride to rats, the highest level of radioactivity, regarding the distribution across the constituents of the blood, was found in the erythrocytes (Khan et al., 1995). Compared to a single dose there was an 85% increase in the iron content in the spleen, but not in the liver, after three doses.

The metabolism of aniline occurs primarily by *N*-acetylation as well as ring- and *N*-hydroxylation in the liver ("non-toxic metabolism"). Animal experiments showed strong interspecies differences regarding the position (*ortho*-, *meta*- or *para*-) of the hydroxyl group, particularly for ring hydroxylation (McCarthy et al., 1985). In humans, most of the aniline is converted by *N*-acetylation to *N*-acetylaniline (acetanilide) and subsequent ring hydroxylation to *N*-acetyl-4-aminophenol (NA4AP, paracetamol) (Figure 2). This can be followed by further conjugation reactions. Glucuronic acid and sulphate conjugates account for the majority of urinary aniline metabolites (MAK, 1992).

The toxic effect of aniline, on the other hand, is based on the oxidative metabolism of aniline via *N*-hydroxylation. The resulting *N*-phenylhydroxylamine can be oxidised in the erythrocytes to nitrosobenzene, simultaneously oxidising the iron(II) of the haemoglobin to iron(III), leading to the formation of methemoglobin (Met-Hb). Met-Hb does not contribute to oxygen transport in the blood. The nitrosobenzene can either react with SH groups of haemoglobin or glutathione to form Hb or glutathione adducts, respectively, or produce further reactive *N*-phenylhydroxylamine in a redox cycle using NADPH. This means that one equivalent of *N*-phenylhydroxylamine can induce the formation of several equivalents of Met-Hb. However, in the case of higher exposure to *N*-phenylhydroxylamine, the consumption of NADPH acts as a limiting factor on the formation of further Met-Hb. Aniline itself, however, is not a direct Met-Hb inducer (Ciccoli et al., 1999).

N-Hydroxylation as the start of the "toxification pathway" competes with both ring hydroxylation and *N*-acetylation as "non-toxic pathways". In a study of occupationally exposed persons, Lewalter und Korallus (1985) demonstrated that the Met-Hb concentration after exposure to aniline is influenced by the rate of *N*-acetylation. Genetic differences in the population regarding enzyme levels (specifically: *N*-acetyltransferases, NAT) could thus lead to individual differences in the toxicokinetics of aniline (Leggett et al., 2021). So-called "slow acetylators" could therefore have an increased risk of aniline-induced health effects (such as methemoglobinemia, see below) (Lewalter & Korallus, 1985). It is believed that "slow acetylators" are also more susceptible to tumours induced by certain carcinogenic aromatic amines (Hein, 2000; Hein et al., 2000; Hein, 2002). However, a more recent human study was unable to find any differences in Met-Hb formation or in the urinary excretion of aniline after a total of six hours (exclusively) inhalation exposure to an aniline concentration of 2 ml/m^3 (= 2 ppm) between the study groups comprising a total of 19 test persons (15 "slow acetylators", 4 "fast acetylators"). (Käfferlein et al., 2014). However, the blood of the "slow acetylators" had approximately 30% higher Hb adduct levels than that of the "fast acetylators". In contrast, a study with oral administration showed differences in the excretion of unconjugated aniline and acetanilide depending on the acetylation status (Modick *et al.*, 2016).

Furthermore, *O*-acetylation of *N*-phenylhydroxylamine by NAT and subsequent acetate cleavage could form reactive nitrenium ions, which could lead to DNA adduct formation. Due to the slightly acidic pH-value of the urine, reactive nitrenium ions could also be formed directly by protonation of *N*-phenylhydroxylamine and subsequent dehydration in the urinary bladder (Figure 2) (Leng et al., 2019). However, there is no evidence in the literature that nitrenium ions are actually formed *in vivo* after administration of aniline.

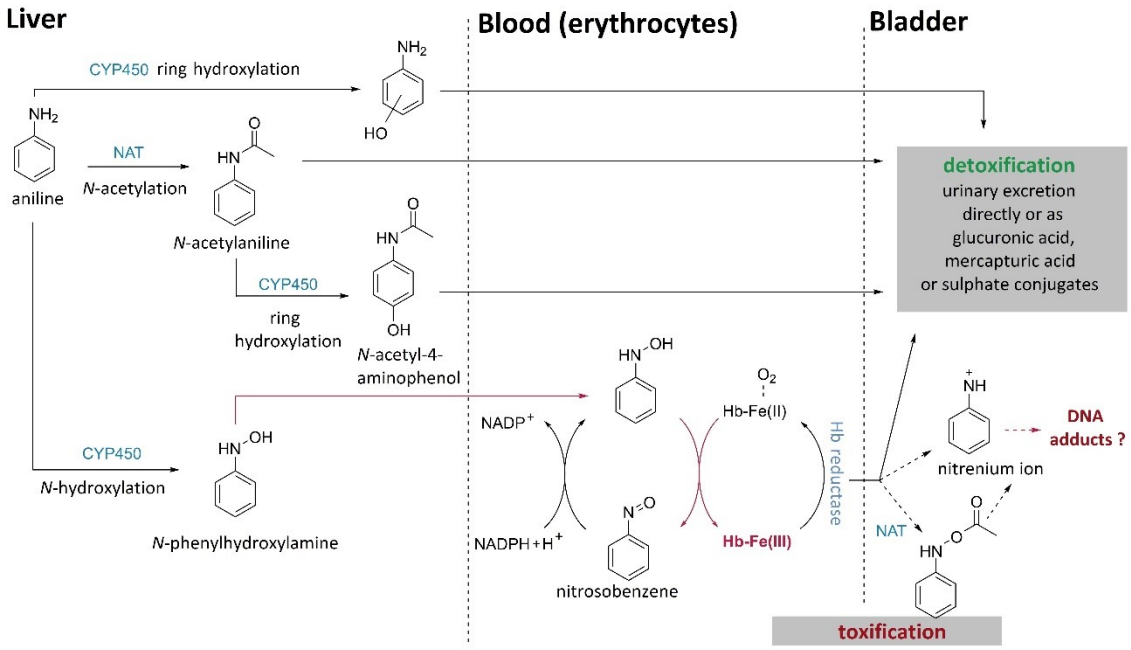

Figure 2: Metabolism of aniline in humans and the enzymes involved (according to Käfferlein et al., 2014). *CYP450*: Cytochrome P450; *Hb*: Haemoglobin; *Hb-Fe(III)*: Methemoglobin; *NADPH*: Nicotinamide adenine dinucleotide phosphate, reduced form; *NADP*$^+$: Nicotinamide adenine dinucleotide phosphate, oxidized form; *NAT*: *N*-acetyltransferase.

Studies using intravenous injection of radioactively (^{3}H and ^{14}C) labelled aniline in rats and mice to investigate the binding of aniline metabolites to macromolecules have shown covalent binding to proteins and RNA and, to a lesser extent, to DNA in the liver, spleen, kidneys, and intestine (Roberts & Warwick, 1966; McCarthy et al., 1985). Compared to mice, rats showed increased radioactivity in all tissues as an indicator of binding to macromolecules. This is explained by changes in aniline metabolism in rats at higher doses, presumably due to an enzymatic saturation effect. This leads to the formation of more reactive metabolites with higher affinities for DNA, RNA and proteins. In contrast, mice are able to maintain their dominant metabolic pathway *via* conjugation with glucuronic acid even at higher doses, whereby ensuring an efficient elimination of the metabolites (McCarthy et al., 1985; MAK, 1992).

Oral administration of ^{14}C-labelled aniline to rats resulted in a predominant urinary excretion, with approximately 90% of the administered radioactivity being eliminated within 24–48 h (McCarthy et al., 1985; MAK, 1992). Human studies with inhalation and dermal exposure to aniline revealed a half-life ($t_{1/2}$) of 3.5 hours for the elimination of 4-aminophenol *via* urine (Piotrowski, 1972, zitiert nach MAK, 1992; Piotrowski, 1977). A more recent human study with oral administration of ring-deuterated aniline (Modick et al., 2016) confirms this value for the main metabolite *N*-acetyl-4-aminophenol (NA4AP; $t_{1/2}$: 3.4 – 4.3 h), while an elimination half-life of 0.6 – 1.2 h was determined for free (non-metabolised) aniline. Overall, an average of ~70% of the dose was excreted as NA4AP (65.2%), NA4AP-mercapturic acid (4%), unconjugated aniline (0.24%) and acetanilide (0.26%) over a period of 48 h post-dose, with other unmeasured metabolites (e.g. 4-aminophenol) probably making up the remaining (unrecovered) ~30%. According to Piotrowski (1957) 4-aminophenol, as well as all metabolites that can be converted into 4-aminophenol by hydrolysis, reflect 13–55 mol% of the dermally absorbed amount of aniline that can be recovered in urine; 4-aminophenol can thus also be used as a biomarker of aniline exposure

(Baranowska-Dutkeiwicz, 1982; Talaska & Al-Zoughool, 2003). In rats, too, 4-aminophenol accounts for about 16% of the aniline metabolite spectrum in urine (Kao et al., 1978).

Mode of action

The most important toxic effect of aniline is damage to the nervous system and erythrocytes, e.g. by the formation of methemoglobin (Met-Hb), known as methemoglobinaemia. This damage is caused by the interaction of *N*-phenylhydroxylamine with O_2-haemoglobin to form nitrosobenzene and Met-Hb (Figure 2). This leads to impaired transport of oxygen to the organs and tissues and can lead to cyanosis (bluish skin discoloration), headache, increased heart rate, dizziness, nausea with vomiting and other symptoms and even death (see e.g. Lubash et al., 1964).

In general, the formation of Met-Hb is reversible due to the activity of erythrocyte Met-Hb reductase; However, if the concentration of Met-Hb exceeds the capacity of Met-Hb reductase, this may lead to the formation of, e.g. hyperoxide anions, a reactive oxygen species, which leads to oxidative stress in the erythrocytes (Jarolim et al., 1990; Grossman et al., 1992; Jollow & McMillan, 2001; Dickinson & Forman, 2002; zitiert nach Koeniget al, 2018). In addition, the redox balance is disturbed by the iron ions released from damaged erythrocytes, which also contribute to oxidative stress (Koenig et al., 2018). In addition, due to the precipitation of haemoglobin in the erythrocytes (Heinz bodies), there is reduced elasticity and deformability, which in turn leads to an accumulation of damaged erythrocytes and reduced degradability in the red pulp of the spleen (Koenig et al., 2018). The associated accumulation of cell debris in the vessels of the spleen causes swelling and inflammatory reactions, which can also result in fibrosis of the spleen. The direct administration of nitrosobenzene to rats also leads to the effects described (Khan et al., 2000). In rats – but not in mice (and hamsters) – this cascade of effects ultimately leads to the observed spleen tumours (National Toxicology Program, 1978; CIIT, 1982; Hecht et al., 1983). The underlying molecular mechanisms are explained in detail in the review article by Makhdoumi et al. (2019).

The observation that tumours are primarily formed in male rats could be due to the males' higher sensitivity (also shown for other chemicals) to iron-mediated carcinogenic effects compared to female rats. In addition, mice (and hamsters) also showed no tumour development, probably due to significantly higher levels of Met-Hb reductase compared to rats (Srivastava et al., 2002).

Non-carcinogenic effects

A single oral or intravenous administration of aniline to male Wistar rats led to methemoglobinemia, the formation of Heinz bodies and an enlarged spleen. The *"No Observed Effect Level"* (NOEL; the highest dose at which no statistically significant treatment-related effect occurs in animal experiments) of this study was 20 mg/kg bodyweight per day for the oral dose. In the same study, volunteers were also exposed to aniline *via* the oral route. For this purpose, 17 male and 3 female persons aged between 22 and 45 years were given 5, 15 or 25 mg aniline orally on three consecutive days. Some of these people received further, higher amounts of up to a maximum of 65 mg aniline on subsequent days. The Met-Hb content in the blood was determined 1, 2 and 3 hours after oral administration. A significant increase was only seen after the administration of doses equal to or higher than 25 mg, so that the authors derived a NOEL of 15 mg, which

corresponds to a dose of 0.21 mg/kg bodyweight for an assumed bodyweight of the test persons of 70 kg. The administration of 35 mg aniline resulted in an increase in the Met-Hb level by 3.7%; at 45 mg this was already 7.08% and at the highest dose of 65 mg it was a maximum of 11.07%, with the level returning to the normal range one hour after the maximum value had been reached. Since Met-Hb levels of up to 5% are considered non-adverse (Henschler & Lehnert, 1986), administration of 35 mg aniline, corresponding to a dose of 0.5 mg/kg bodyweight (when assuming a bodyweight of 70 kg), can be regarded as the *"No Observed Adverse Effect Level"* (NOAEL; the highest tested dose at which no adverse/harmful effect is observed) of this study.

In a subacute study with male F344 rats, which were given aniline hydrochloride in doses of 10, 30 and 100 mg/kg bodyweight per day (the amounts actually ingested were at least 4, 12 and 41 mg aniline/kg bodyweight per day) *via* food, effects on the haematopoietic system due to the formation of Hb adducts were seen in 2 out of 6 animals of the lowest dose group after just one week (Mellert 2004; Zwirner-Baier 2003). A *"Lowest Observed Adverse Effect Level"* (LOAEL; lowest dose in a toxicological study in which an adverse substance-related effect was observed) of 4 mg/kg bodyweight per day for the formation of Hb adducts can therefore be derived from this study.

A 2-year study with oral administration of aniline hydrochloride *via* food (corresponding to 0, 7, 22 and 72 mg aniline/kg bodyweight per day) in F344 rats (CIIT, 1982) yielded a LOAEL of 7 mg/kg bodyweight per day for systemic, non-neoplastic effects. The animals of the lowest dose group showed, amongst others, an excessive accumulation of iron deposits (haemosiderin) in the spleen and increased haematopoiesis in the red pulp of the spleen, a significantly increased number of young, still immature red blood cells (reticulocytes) with a simultaneous reduction in the number of mature erythrocytes. Since these effects already occurred in the lowest dose group, a NOAEL could not be derived.

In a study on prenatal and postnatal developmental toxicity, pregnant F344 rats were administered aniline hydrochloride (corresponding to 7, 22 and 72 mg aniline/kg bodyweight per day) by gavage from the 7th to the 20th day of gestation (GD 7 to GD 20) or from GD 7 to the end of pregnancy. Amongst others, foetuses of the highest dose group showed increased relative liver weights and significantly enlarged erythrocytes with a reduced size distribution range; the NOAEL for foetal effects was 22 mg/kg bodyweight per day. However, the offspring of the low dose group had transiently higher relative liver weights on postnatal day 25 (PND 25) and PND 50, but not on PND 60. The study also revealed no evidence of embryolethal or teratogenic effects even at maternally toxic doses (Price et al., 1985).

In addition to the effects in the spleen, aniline is subject to a harmonised classification as a Category 1 skin sensitiser (Skin. Sens. 1); various tests indicate that aniline has a sensitising potential and can lead to an allergic skin reaction (contact dermatitis) (ECB, 2004).

The risk assessment in this Opinion is based on the LOAEL of 4 mg/kg bodyweight per day from the subacute study as the lowest toxicological reference value for the non-carcinogenic effects.

Genotoxicity

Numerous *in vitro* gene mutation studies in bacteria and mammalian cells as well as *in vitro* and *in vivo* cytogenicity studies are available for aniline. The available data and the

genotoxic potential of aniline have been discussed intensively in the scientific literature and committees (ECB, 2004; Bomhard & Herbold, 2005; MAK, 2007; IARC, 2020).

While bacterial gene mutation tests (standard *in vitro* Ames test) are negative, positive results are reported in *in vitro* mammalian cell gene mutation tests, i.e. the mouse lymphoma assay TK+/- (MLA) (Mitchell et al., 1988; McGregor et al., 1991).

In *in vivo* studies in rats, high doses of aniline resulted in a slightly increased incidence of chromosome aberrations and micronuclei in immature (polychromatic) bone marrow erythrocytes (Bomhard, 2003). Micronuclei were also induced in the mouse, but no chromosomal aberrations in the bone marrow (Jones & Fox, 2003). In a test with incubated chicken eggs (hen's egg test - micronucleus induction, HET-MN), the administration of aniline during a developmental phase of increased blood formation in the yolk sac membrane, which is also a site of biotransformation, led to an increase in the micronucleus rate (Reisinger et al., 2021). A study on transgenic (BigBlue) rats given 100 mg/kg bodyweight per day by gavage showed increased micronucleus rates in reticulocytes, haematological effects including Met-Hb formation, and increased spleen and liver weights, but no increased mutation frequencies in the liver tissues, spleen and spinal cord (Koenig et al., 2018). Due to the simultaneously detected significant reduction in the number of erythrocytes and the increase in the number of reticulocytes and in the Met-Hb level, the authors conclude that the micronucleus formation is due to compensatory effects, i.e. to an increased cell renewal as a result of the erythrocyte toxicity caused by aniline. Furthermore, the treatment of incubated chicken eggs and subsequent analysis of the embryonic chicken liver using the comet assay and ^{32}P-postlabeling for the detection of DNA adducts did not provide any evidence of DNA reactivity of aniline (Koenig et al., 2018; Kobets et al., 2019).

Carcinogenicity

At high doses, orally administered aniline induces tumours of the spleen in rats (mainly males) (National Toxicology Program, 1978; CIIT, 1982), but not in mice (National Toxicology Program, 1978) and hamsters (Hecht et al., 1983). In the study by the Chemical Industry Institute of Toxicology (CIIT), F344 rats (130 animals per sex and group) were given aniline hydrochloride in doses of 0 (control), 7, 22 and 72 mg/kg bodyweight per day over a period of 104 weeks, administered orally via the feed. While no tumours developed in either the female or the male animals in the controls and in the lowest dose group, one male in the medium dose group developed spleen tumours. In the highest dose group (72 mg/kg bodyweight per day), 35 males (and one female) developed spleen tumours. This study was also used to derive the LOAEL for non-carcinogenic effects of 7 mg/kg bodyweight per day (see above), i.e. the spleen tumours observed only occurred above doses at which severe damage to the erythrocytes was already observed. Overall, the available data indicate an organ-, species- and sex-specific pattern of carcinogenesis with a non-linear, very steep dose-frequency relationship for tumour formation in the spleen of male rats.

Some experts and committees suspect a causal relationship between erythrocyte toxicity, induction of iron-mediated oxidative stress, increased erythropoiesis and DNA damage, which can ultimately lead to tumour formation (u. a. Wu et al., 2005; Wang et al., 2008). Various authors thus postulate the existence of a threshold dose below which the described reaction cascade, which ultimately leads to the tumours in the rats' spleen, is not triggered (e. g. Bus & Popp, 1987; Mellert et al., 2004; MAK, 2007; SCOEL, 2010; SCOEL, 2015; MAK, 2018).

In various cohort studies on employees of the chemical industry which were exposed, *inter alia*, to aniline, the increased rate of observed bladder cancer could not be unambiguously attributed to aniline (SCOEL, 2015; MAK, 2018). Rather, the increased incidences in those occupationally exposed were attributed to the simultaneous exposure to other substances, above all *o*-toluidine, which induces bladder cancer *via* the formation of DNA-binding nitrenium ions (Carreon et al., 2010; Carreon et al., 2014; Tajima et al., 2020; Park et al., 2021). This interpretation is supported by a chronic study in F344 rats with oral administration of aniline and other aromatic amines such as e.g. *o*-toluidine *via* food (Goodman et al., 1984). In contrast to *o*-toluidine, the sole administration of aniline alone neither led to the formation of γ-H2AX (phosphorylated histone as a general biomarker for e.g. DNA damage) nor to histopathological changes in the urinary bladder epithelium (Toyoda et al., 2019).

In contrast, the International Agency for Research on Cancer (IARC) classified aniline as "probably carcinogenic to humans" (Group 2A) in June 2020 (DeMarini et al., 2020; IARC, 2020). The basis for this were, *inter alia*, mechanistic considerations that aniline belongs to a group of aromatic amines, some of which have been classified by IARC as known carcinogens to humans (Group 1 carcinogens). In addition to these mechanistic considerations, aniline and aniline hydrochloride were tested for the presence of various key characteristics of carcinogens, including the induction of protein/haemoglobin and/or DNA adducts, genotoxicity or the induction of oxidative stress.

Using the benchmark dose approach (EFSA, 2007; EFSA, 2018), the European Food Safety Authority (EFSA) determined an effect dose ($BMDL_{10}$) of 29 mg/kg bodyweight per day for a 10% increase in tumour incidence, based on data on the frequency of spleen tumours in male rats from both carcinogenicity studies (National Toxicology Program, 1978; CIIT, 1982). The US Environmental Protection Agency (EPA) also takes the view that in the absence of reliable evidence for a threshold value, it must be assumed that even the smallest amounts of aniline could have a tumour-inducing effect; using the linearised multistage model, the US EPA derived an oral cancer slope factor (CSF) of 5.7×10^{-3} per mg/kg bodyweight per day (US EPA (2000)). The study on which the calculation of the CSF is based is the 2-year study in rats (CIIT, 1982).

The risk assessment for (potential) carcinogenic effects in this Opinion is therefore based on using the CSF of 5.7×10^{-3} per mg/kg bodyweight per day, being the most conservative approach.

3.1.3 Exposure assessment for free aniline

Finger paints are products intended for intensive skin contact, some of which are even intended for children below 36 months of age; most of the products for children under 36 months are designated for the age group from 2 years on ("2+") (CEN ISO/TR 8124-8:2016), but a few are also for children from 12 months on.

Due to the mouthing behaviour (putting objects in the mouth) of small children, in addition to dermal exposure through intended use, (unintentional) oral uptake must also be assumed for this age group.

Determination of total exposure to finger paints

The BfR does not have any reliable usage data for the exposure assessment, therefore a very conservative approach is used in the following.

According to the exposure scenario for finger paints in the Children's Toys Fact Sheet (RIVM, 2002), an 18-month-old, 9.85 kg child plays with finger paints 100 times a year for an average of 45 min each. Contact with the paint occurs primarily with the inner side of the hands, resulting in intentional dermal exposure. In addition, according to the *Toys Fact Sheet*, it is assumed that 30 mg of finger paint is swallowed per minute through hand-to-mouth contact, which results in a total ingestion of 1,350 mg per play event (45 min × 30 mg).

However, when calculating cancer risk, not just a single point in time is relevant, but the additional risk summed up over the entire lifetime (lifetime excess cancer risk, LCR). Therefore, in a conservative approach, it was assumed that a child would play regularly with finger paints, starting from the age of one year up to the age of 14 (analogous to the scope of the EU Toy Safety Directive), having contact with the inner sides of the hands and, depending on the age, swallowing either 400 mg (children up to 36 months) or 0 mg (children over 36 months).

Since finger paints are now obliged to contain bitter substances according to EN 71-7, the BfR assumes an oral intake of 400 mg for children up to 36 months of age (based on the opinion of (SCHER, 2016) on liquid/sticky toy materials) instead of the 1350 mg suggested by the Dutch National Institute for Public Health and Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM), while no oral intake is assumed for children over 36 months. Also deviating from the exposure scenario of the RIVM, the BfR is of the opinion that children increasingly use other products for creative crafting as they get older, so that a reduced frequency of playing with finger paints of 12 times a year is assumed for children above the age of 36 months. The age-dependent bodyweights, dermal exposure areas, oral intake levels, and frequency of play are summarised in Table 2.

Table 2: Exposure parameters for estimating dermal and oral uptake of aniline via finger paints for different age groups of children.

Age (years)	Bodyweight (kg)	Dermal exposure area		Oral intake amount	Play frequency (per year)
		hand size (cm^2)	Inner side (cm^2)	Finger paint (mg)	
1-2	9.8	260	130	400	100
2-3	12.4	270	135	400	100
3-6	15.7	330	165	0	12
6-11	24.3	460	230	0	12
11–14*	44.8	640	320	0	12

*Bodyweight and hand size values are for 11–16 year old children. However, the Toy Safety Directive only applies to toys for children up to 14 years of age, so the exposure calculation was only carried out for children up to 14 years of age

For the dermal exposure assessment in this Opinion, the average absorption rates of 320 µg/cm^2/h, determined in the *in vivo* test for an aqueous solution with an aniline concentration of 1% and a contact time of 30 min, and 200 µg/cm^2/h for a contact time of 60 min (see 3.1.2) were averaged and linearly interpolated to an assumed playing time of 45

min, which resulted in an absorption rate of 260 µg/cm^2/h. For the sake of simplicity, it was assumed that the absorption rate determined for aqueous solutions also applies to viscous finger paints. Depending on age, this resulted in systemic exposure doses (SED_{derm}) between 1.39 µg/kg bodyweight per event (for 11 to 14-year-old children) and 2.59 µg/kg bodyweight per event (for 1- up to 2-year-old children) (see Table 3).

For oral exposure, assuming that in humans the resorption of aniline from finger paints in the gastrointestinal tract is 100% (see above), the systemic exposure doses (SED_{oral}) are in the range between 0 and 0.41 µg/kg bodyweight per event.

The total exposure (SED_{total}) is calculated as the sum of the dermally and orally absorbed amounts (SED_{derm} + SED_{oral}) and lies between 1.39 µg/kg bodyweight per event (for 11 to 14 year olds) and 2.99 µg/kg bodyweight per event (for 1 to 2 year olds).

With a playing frequency of 100 and 12 times per year, respectively, these event-related exposure doses can be extrapolated to yearly average daily doses (YADD = SED × (100 or 12)/365, analogous to lifetime average daily dose according to US EPA (2005a)). An additional LCR can then be determined for each age-related YADD, which is the product of the oral cancer slope factor (CSF) of 5.7×10^{-3} (mg/kg bodyweight per day)$^{-1}$ (based on the observation of spleen tumours after chronic oral exposure of rats according to US EPA (1988)) and the YADD. The LCR calculation also takes into account a factor for increased sensitivity of children to mutagenic carcinogens without a threshold value in the early years of life (age-dependent adjustment factor, ADAF); due to the lack of substance-specific information, the BfR uses the standard values specified by the US EPA (2005b): 10 for children up to 24 months, 3 for older children up to < 16 years, and the duration of the considered phase of life in relation to an assumed life expectancy of 70 years.

3.1.4 Risk characterisation

Non-carcinogenic (systemic) effects

The concept of the margin of safety (MoS) is used to assess the non-carcinogenic effects. The MoS corresponds to the ratio of a health-based reference value and the systemic exposure dose SED_{total}. Since the hazard characterisation provides a LOAEL (lowest dose with observed adverse effects used in animal studies) as a health-based reference value, the MoS should be at least 300. This value results from multiplying the uncertainty factors for the intra- and interspecies-specific differences in toxicokinetics (factor 10) and toxicodynamics (factor 10) and the fact that the LOAEL is used instead of a NOAEL (factor 3). The subacute study with dietary administration of aniline to rats (Zwirner-Baier et al., 2003; Mellert et al., 2004) revealed effects on the haematopoietic system at a dose of 4 mg/kg bodyweight per day (LOAEL) after just one week of intake. Taking into account an oral bioavailability of (at least) 89% for aniline in the rat, the systemic LOAEL is 3.56 mg/kg bodyweight per day. The resulting MoS values are therefore between 1,336 (1- to 2-year-olds) and 2,872 (11- to 14-year-olds) and can thus be considered as sufficiently large even assuming daily play frequency.

Accordingly, with a content of 10 mg/kg free aniline in finger paints, the probability of health impairments occurring with regard to the non-carcinogenic systemic effects is very low, even with aggregated exposure through simultaneous oral and dermal absorption.

Carcinogenic effects (assumption of a genotoxic mechanism of action without a threshold value)

Although the available data indicate an indirect mechanism of tumour development in rats, the absence of a threshold value cannot be ruled out with certainty. In 2010, SCHER recommended the concept of the additional LCR as the only acceptable approach for the assessment of carcinogens in toys for which no threshold value can be derived (SCHER, 2010). An additional tumour incidence of 1×10^{-6}, i.e. one additional case of cancer for every million people exposed to this dose, was suggested by SCHER as an acceptable risk for adults, while an additional safety factor may be necessary for children. Therefore, under the (conservative) assumption of a non-threshold mechanism of action, the following equation is used to calculate the associated additional LCR:

$$\text{additional LCR} = \text{CSF} \times \text{ADAF} \times \text{YADD} \times \text{TF}$$

whereby

CSF: cancer slope factor, here: 5.7×10^{-3} per mg/(kg bodyweight per day) US EPA (2005b)

ADAF: age dependent adjustment factor, here: 10 for 0 – <2 years, 3 for >2 – <16 years

YADD: year adjusted daily dose, see Table 3

TF: time fraction, here: Number of years in age group / 70 years

The additional LCR is first calculated separately for each age group (see Table 3). The individual LCR values are then summed across all age groups. Taking into account the underlying exposure scenario, the additional LCR of 0.99×10^{-6} from playing with finger paints with an aniline concentration of 10 mg/kg lies in the range of 1×10^{-6} considered acceptable according to SCHER.

Table 3: Systemic exposure doses for dermal (SED_{dermal}), oral (SED_{oral}) and total intake (SED_{total}) as well as the yearly average daily dose (YADD) calculated on the basis of the frequency of use of finger paints (100 or 12 × per year). The additional lifetime cancer risk (LCR) and the margin of safety (MoS) with regard to systemic effects from playing with finger paints, each containing 10 mg free aniline/kg, result from the YADD and SED_{total}, respectively.

Age (years)	**SED_{dermal}**	**SED_{oral}**	**SED_{total}**	**YADD**	**Additional LCR**	**MoS**
	(µg/kg bodyweight/event)			(µg/kg bodyweight/day)		
1-2	2.59	0.41	2.99	0.82	0.67×10^{-6}	1,336
2-3	2.12	0.32	2.45	0.67	0.16×10^{-6}	1,636
3-6	2.05	0	2.05	0.07	0.05×10^{-6}	1,952
6-11	1.85	0	1.85	0.06	0.07×10^{-6}	2,167
11-14	1.39	0	1.39	0.05	0.03×10^{-6}	2,872
					Total: 0.99×10^{-6}	

3.1.5 Uncertainty analysis

The risk assessment presented here involves several uncertainties. On the one hand, there are still data gaps regarding the toxicology of aniline. Until now it has not been proven

beyond doubt whether the carcinogenic effect shown in the (male) rat is subject to a threshold mechanism and how or whether these findings can be extrapolated to humans.

Further uncertainties exist due to the limited/incomplete data on the use of finger paints by children and thus in the parameters that were used to calculate exposure to aniline from finger paints. There are uncertainties in the absorption rate used for absorption through the skin and in relation to the body area in contact with the finger paint, the assumed frequency of play and the amount orally ingested per age group. Beyond that, extrapolating the systemic exposure dose for a day on which children play with finger paints to days on which no exposure takes place is only legitimate for substances that show a linear dose-response relationship. Whether aniline is one of these has not yet been conclusively clarified.

In view of the identified uncertainties, the risk assessment was designed in such a way that, whenever possible, each parameter was chosen such that the resulting exposure assessment tends to lead to an overestimation of the actual exposure. In terms of the hazard potential, too, the most conservative option for the endpoint carcinogenicity was chosen by assuming the absence of a threshold value, so that all other options (lower exposure, mode of action with a threshold value) are also included. Since a health risk is not to be expected even for these very conservative assumptions regarding exposure and hazard potential, the probability of health impairments from finger paints with free aniline concentrations at the level of the limit value can be regarded as very low.

3.2 Other aspects

In the health risk assessment presented here, it was assumed that every time a child plays with finger paint, it contains aniline at the maximum permissible amount of 10 mg/kg. However, a query to the state investigation offices revealed that such high concentrations have not been detected in products so far. The maximum analysed concentration in approx. 60 finger paints measured within the period from 2015 to the end of 2020, was 8.6 mg aniline/kg. This value was measured in a yellow finger paint (only) after reductive cleavage. In this case, a concentration of up to 30 mg/kg would be permissible, which is targeted toward a ban on the use of azo dyes that can release aniline after reductive cleavage. Another yellow dye contained 4.3 mg/kg, a red dye 5.7 mg/kg, both also only after reductive cleavage. The aniline concentration of all other samples, both with and without prior reductive cleavage, was below the detection limit of 2 mg/kg (when analysed using gas chromatography with tandem mass spectrometry; GC-MS/MS) or 0.8 mg/kg (when analysed using liquid chromatography with diode array detector; LC/DAD). The actual additional lifetime cancer risk is therefore very likely to be significantly lower than the risk level of 1×10^{-6} deemed acceptable according to SCHER.

Further information on the subject of toys from the BfR website
Subject page on the health assessment of toys:
https://www.bfr.bund.de/en/health_assessment_of_toys-54510.html

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