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Coolants in e-cigarettes are poorly researched: health impairments possible

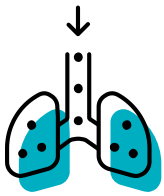
There is a lack of data, especially regarding possible effects on the lungs during inhalation

→ Updated the version of 26 March 2025

In brief

- Synthetic coolants such as WS-23, WS-3 and WS-5 are widely used in e-cigarettes. They are added to the liquids that are vaporised and inhaled when consuming e-cigarettes.
- The coolants cause a cooling sensation when users inhale the vapour, which consumers often find pleasant.
- As the cooling effects make it easier to inhale, they can result in increased nicotine intake and possibly lead to greater dependence, especially among young and inexperienced users.
- The data available on the potential health risks of the substances is very limited. In particular, the effects of inhalation (inhalation toxicity) have been poorly investigated.
- The German Federal Institute for Risk Assessment analysed the available scientific data (from cell and animal studies) on these three coolants and assessed the health risks. They found that, when using e-cigarettes, the amounts of the substances that enter the body are higher than the amounts for which **no** health effects occurred in animal tests. In the opinion of the BfR, a health risk for consumers is therefore to be expected, especially with regular consumption.
- In the animal studies, absorption of the substances into the body primarily led to damage to the liver and kidneys. It is currently not possible to say how the substances affect the lungs when inhaled, as there have been no studies on this.

How do coolants from e-cigarettes enter the body?

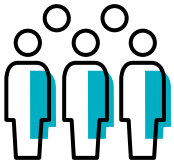


Coolant intake occurs via **inhalation** when using e-cigarettes. In order to assess the health risks for the present opinion, the researchers operated under the assumption that the inhaled substances are completely absorbed by the body.

Is there a health based guidance value?

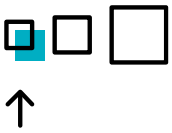
No. A health-based **guidance value** describes the intake amount of a given substance for which no health risk for consumers is to be expected according to the available data. To date, there is no such guidance value for the coolants under consideration. The researchers have derived a different value from the available data, the NOAEL (no-observed-adverse-effect level). The NOAEL indicates the highest tested dose of a substance at which no health damage was observed in tests. The value was used as the basis for calculating other parameters that can be used to estimate potential health risks.

Is there a health risk?



Impairment to the health of the **general population** when **using e-cigarettes** is probable, especially with regular consumption of liquids with high concentrations of coolants. In animal experiments, damage to the liver and kidneys in particular was observed.

How high-quality is the data?



The quality of the data is **low**. In particular, no relevant data are available on the effects of the substances on the lungs when inhaled.

How can the health risk from posed by coolants in e-cigarettes be reduced?

The government can ban the use of coolants in e-cigarettes.

Manufacturers can dispense with the addition of coolants in e-cigarettes or reduce the concentration.

Consumers can refrain from consuming e-cigarettes or liquids with coolants.

1 Subject of the assessment

In the present opinion, a health risk assessment of substances in e-cigarettes or refill liquids (e-liquids) that produce a cold sensation (so-called cooling agents, hereinafter referred to as coolants) was carried out.

This assessment focuses on the coolants N,2,3-trimethyl-2-iso-propyl-butyramide (WS-23; CAS 51115-67-4; EC 256-974-4), N-ethyl-2-isopropyl-5-methylcyclohexane-carboxamide (WS-3; CAS 39711-79-0; EC 254-599-0) and ethyl 2-[[(1R,2S,5R)-5-methyl-2-propan-2-ylcyclohexanecarbonyl]amino]acetate (WS-5; CAS 68489-14-5; EC 695-735-2). The assessment was prompted, among other things, by the widespread use of coolants in e-liquids as well as by a report from Baden-Württemberg of a case of serious and ultimately fatal lung damage after consuming an e-cigarette containing WS-23. The concentrations of WS-23 in products on the market vary greatly in some cases. The responsible authority (Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe, CVUA-OWL) reports WS-23 concentrations between 0.55 and 0.86% by weight in analysed disposable e-cigarettes . In the EU Common Entry Gate (EU-CEG), concentrations of up to 4% by weight have been reported by the importer.

Opinion No. 045/2015 of the German Federal Institute for Risk Assessment (BfR), issued on 30 July 2015, assesses the health risks of additives in tobacco products and e-cigarettes, including WS-23, WS-5 and WS-3. Therein, the BfR recommends a comprehensive risk assessment for inhaled coolants and, in the context of the cooling and local anaesthetic effects (which facilitate inhalation of tobacco smoke), suggests that groups of substances with TRPM8 receptor agonists, such as substituted p-menthane compounds, should be examined and possibly banned, as they are expected to facilitate inhalation in a similar way to menthol. This has also been done for conventional smoking tobacco in the form of Annex 1 of the German Tobacco Products Ordinance (TabakerzV) due to the inhalation-facilitating properties of these substances.

The coolants WS-23, WS-3 and WS-5 are widely used in e-cigarettes and give consumers a cooling sensation which is often perceived as pleasant. Originally, these compounds were developed as part of a research programme to find refreshing substances for shaving products. Today, they are used in various products, including e-liquids, in which they simulate a cooling effect without affecting flavour. They act on the sensory nervous system, specifically on the TRPM8 receptor (Transient Receptor Potential Melastatin 8), which is responsible for the perception of cold. This receptor is normally activated by low temperatures, but also by agonists such as menthol, which signals cooling to the brain. Cooling agents such as WS-23, WS-3 and WS-5 are also TRPM8 receptor agonists and produce this effect without actually lowering temperature.

Although coolants such as WS-23, WS-3 and WS-5 are already used in various products, there is insufficient data on the inhalation toxicity of these substances, particularly in the context of e-cigarettes. Previous toxicological assessments of these substances are mainly based on oral or dermal exposure scenarios. As e-cigarettes allow direct inhalation of aerosols, respiratory exposure is of particular importance. There is thus an urgent need for specific data on inhalation toxicity and possible long-term health effects.

In this risk assessment for the coolants WS-23, WS-3 and WS-5, the Margin of Exposure (MOE) approach was used and a Derived No Effect Level (DNEL) was determined. Based on the DNEL, a risk characterisation ratio (RCR) was performed to assess the health risk of inhalation exposure. For each of the substances mentioned, the dose at which no toxicological effects are observed (No Observed Adverse Effect Level, NOAEL) was derived from relevant toxicity studies and compared with the actual exposure. The resulting MOE values show whether the current intake of the substance is likely to cause impairment to health. A low MOE value or falling below a predetermined MOE indicates an increased health risk. For substances that are neither genotoxic nor carcinogenic, the minimum value of an MOE is usually 100 or above. If this value is not reached, a hazard to human health cannot be ruled out (EFSA, 2023). In the present risk assessment, a safety margin of 200 was used for WS-23 and WS-5 in order to take into account the uncertainty factor of 2 for the transfer of results from a subchronic study to chronic exposure and thus better cover long-term effects. For WS-3, an uncertainty factor of 6 was applied (= MOE of 600) to extrapolate from subacute exposure (28 days) to chronic exposure (EFSA, 2012a; ECHA, 2012).

The MOE is not a health-based guidance value, i.e. it is not a safety threshold below which daily intake is considered safe. Rather, the MOE is used when there are indications of harmful effects, but the available information is not sufficient to derive what amount of the substance can be ingested daily without causing effects on human health.

For the sake of comparison, the DNEL was also calculated in accordance with REACH Regulation (EC) 1907/2006. The DNEL represents the derived exposure level below which the substance – taking into account all known uncertainties – leads to no impairment of human health. Furthermore, for risk characterisation, the RCR was calculated as the ratio between the estimated exposure and the DNEL. If the estimated exposure is above the DNEL (RCR ≥ 1), this indicates that a health risk is present (ECHA, 2016).

2 Results

Overall, the general data available on the coolants WS-23, WS-3 and WS-5 is very limited. In particular for the inhalation toxicity of these substances, there are no relevant, valid data. However, the available information on systemic toxicity after repeated oral administration indicates a potential health risk. As there are no relevant data on inhalation intake, 100% systemic availability is assumed. This assumption is based on a worst-case scenario due to the existing data gaps. EFSA reports hepatic and renal toxicity from 10 mg/kg bw (NOAEL: 5 mg/kg bw/day) for WS-23, mild hepatic and renal toxicity from 40 mg/kg bw (NOAEL: 8 mg/kg bw/day) for WS-3, and renal changes, cardiomyopathy in female rats from 675 mg/kg bw and haematological effects from 225 mg/kg bw (NOAEL: 75 mg/kg bw/day) for WS-5.

The evaluation of the available data shows that the reference MOE is not reached and the RCR of 1 is exceeded for the three substances under consideration, WS-23, WS-3 and WS-5, in all exposure scenarios analysed, thus indicating a health risk for users. A comprehensive exposure assessment for these substances suggests that their inhalation via e-cigarettes exceeds exposure levels below which no health effects would be expected based on animal experiments. In particular, regular consumption of e-liquids with high concentrations of WS-23, WS-3 and WS-5 are expected to pose a risk to human health. For this reason, the use of e-liquids containing WS-23, WS-3 and WS-5 is not recommended from a toxicological point of view.

Studies have shown that synthetic coolants such as WS-23 can significantly improve the sensory experience when consuming e-cigarettes and increase the attractiveness of these products (Tackett et al. 2023). These compounds exert their effect via TRPM8 receptors, which are responsible for the perception of cooling effects. Synthetic coolants can also have analgesic and anti-irritant properties. This effect can lead to increased consumption of these coolants. Higher concentrations of coolants in e-liquids in turn lead to increased exposure, which – especially with repeated use – leads to a health risk.

In addition, the apparent cooling effects of WS-23, WS-5 and WS-3 can also lead to an increased intake of nicotine, a change in inhalation behaviour, and possibly to increased dependence, especially among young and inexperienced users.

A comprehensive toxicological assessment of coolants in general is not possible due to the structural differences between the individual substances, so no generally valid statements can be made for all coolants currently available on the market.

3 Assessment

3.1 Risk assessment

3.1.1 Hazard identification

This opinion is limited to the use of coolants in e-cigarettes and e-liquids. Exposure to these substances may also occur via other products, such as cosmetics and food.

WS-23

Among the coolants used in e-cigarettes, the substance WS-23 has received particular attention, as it is the most widely used and has been linked to an incident in Baden-

Württemberg in which a person died of severe lung damage. No evidence could be found for a causal link between the use of WS-23 and the death. However, the substance was detected in high concentrations in e-cigarettes similar to the product used by the person who died.

Chemical properties and structure: WS-23 is an aliphatic carboxylic acid amide that remains stable under various pH and temperature conditions due to its stable chemical structure. Its structure consists of a 2-isopropyl-N,2,3-trimethylbutanamide backbone, which gives it its cooling properties. Unlike menthol, WS-23 has a cooling effect without having a strong taste or odour of its own, which makes it particularly popular in products where the taste should not be affected.

WS-3

Chemical properties and structure: WS-3 belongs to the group of synthetic coolants based on substituted carboxylic acid amides. WS-3 is also chemically stable and is characterised by a different intensity and duration of the cooling effect. It is often used in combination with other coolants such as WS-23 to enhance and prolong the cooling sensation in products.

WS-5

Chemical properties and structure: WS-5 also belongs to the substituted carboxylic acid amides; its chemical name is ethyl 2-[[(1R,2S,5R)-5-methyl-2-propan-2-ylcyclohexanecarbonyl]amino]acetate. In contrast to WS-3 and WS-23, WS-5 leaves a stronger and deeper feeling of cold.

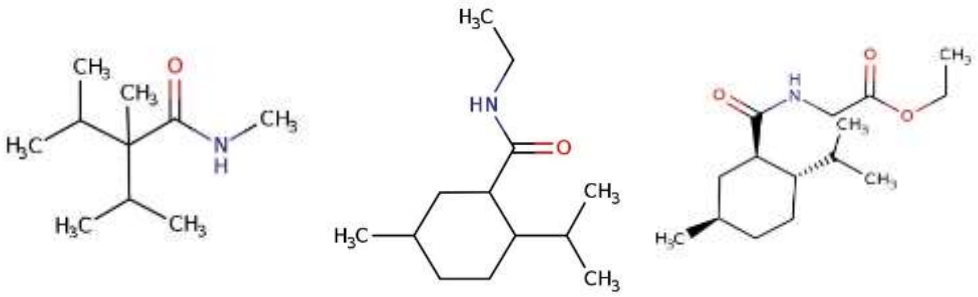


Fig. 1 WS-23, CAS number: 51115-67-4 WS-3, CAS number: 39711-79-0 WS-5, CAS number: 68489-14-5

An excerpt from the EU Common Entry Gate (EU-CEG) for providing information on tobacco products, e-cigarettes and refill containers revealed concentrations of the assessed coolants in e-liquids between 1 and 4%. In addition, it is possible to purchase concentrated coolants for the independent production of e-liquids, which may increase the risk of exposure through the use of high doses.

3.1.2 Hazard characterisation

The coolants WS-23, WS-3 and WS-5 have similar toxicological profiles. However, there are only a few studies that deal specifically with the inhalation of WS-23, WS-3 and WS-5, which makes a comprehensive toxicological assessment of these compounds difficult, especially with regard to inhalation toxicity.

To collect available data, extensive bibliographical literature research was conducted in various databases and search engines, including PubMed, Scopus, Web of Science, Embase, Science Direct, Wiley, Cas SciFinder, OECD Echem Portal, FDA, WHO Iris, PubChem and Google Scholar. In addition, tools such as the OECD QSAR Toolbox and the EFSA Chemical Hazards Database: OpenFoodTox 2.0 were used. Furthermore, data shared by the German Federal Office of Consumer Protection and Food Safety (BVL) and studies from industry, including those from Chinese and US e-cigarette manufacturers, were also considered. The database of the European Chemicals Agency (ECHA; https://chem.echa.europa.eu/) was also consulted.

The presentation published online by a company on a 90-day study with WS-3 and WS-23 in rats was noted (CORESTA, 2023), but the underlying study is not available to the BfR and the information in the published presentation is not sufficient for a toxicological assessment. For these reasons, this study cannot be considered in the assessment.

While the toxicological profiles of the coolants to be assessed have been relatively well studied with regard to oral and dermal exposure, there is a lack of comprehensive data on inhalation toxicity, which is crucial for the assessment of their use in e-liquids. The data available on the individual substances is summarised below.

WS-23

WS-23 is assessed as harmful if swallowed according to CLP Regulation (H302, Signal word: Warning, Acute Tox. 4) according to the classification carried out by companies in the context of REACH registrations. The list of self-classifications in ECHA's C&L database contains a self-classification of WS-23 as harmful if inhaled (H332, Signal word: Warning, Acute Tox. 4). The data-driven reasoning for this self-classification cannot be determined from the available data.

In vitro

With regard to the potential lung toxicity of WS-23, there are current *in vitro* studies that show various adverse cellular effects of the substance. For example, Omaiye et al. (2022) investigated the cytotoxicity and the influence of WS-23 on cell growth and cell morphology in human bronchial epithelial cells. It was found that WS-23 was cytotoxic at the concentrations tested and had a negative effect on the growth of the lung cells tested in a concentration-dependent manner. In addition, WS-23 had an effect on the morphology of the lung epithelial cells in cell culture at the two highest concentrations tested (3% and 10%). In a study by Wong et al. (2023) using 3D lung tissue models, WS-23 was shown to impair the cytoskeleton of lung epithelial cells, in particular by a concentration-dependent reduction of F-actin. This led to inhibition of cell motility and inhibition of the formation of cell-cell junctions (tight junctions). These results suggest that WS-23 in e-cigarettes could exert negative effects on the bronchial epithelium and thus impair human health. Another *in vitro* study by Manevski et al. (2022) shows that the synthetic coolant WS-23 modulates the responses of airway epithelial cells differently. The authors pointed out that WS-23 and nicotine aerosols can induce goblet cell hyperplasia, which could impair airway physiology and increase susceptibility to respiratory diseases.

In vivo

In the case of WS-23, there is particular concern about *in vivo* genotoxicity and possible renal effects (FAO & WHO, 2016).

Acute and subacute toxicity: The acute toxicity of WS-23 is well documented in relation to acute exposure (FAO & WHO, 2009; https://chem.echa.europa.eu/). Based on an acute oral toxicity study in rats, an LD50 (dose at which 50% lethality occurs) of approx. 500 mg/kg bw was determined, resulting in a classification according to CLP as Acute Tox. 4, H302. However, the data available for acute and subacute inhalation exposure is limited. Wu et al. (2021) conducted a study on the acute and subacute inhalation toxicity of WS-23 in Sprague-Dawley rats on behalf of the e-cigarette industry. Neither mortality nor behavioural changes occurred during acute exposure to 340 mg/m³. In a subacute study (342.85 mg/m³ over 28 days), no significant health impairments were observed, neither in terms of body weight or organ function, nor in histopathological examinations. The results indicate that WS-23 has no significant toxicological effects after repeated inhalation of these concentrations. It must be pointed out that the studies – even if they are described in the publication as conforming to the test guidelines – were not conducted in accordance with current and validated test guidelines. For example, the selected test concentrations do not correspond to the specifications of the OECD 403 and 412/413 test guidelines applied according to the authors. For an acute inhalation study, OECD test guideline 403 (OECD, 2024) specifies that the animals are to be exposed to a very high concentration termed the limit concentration, provided that it can be assumed that the substance is not acutely toxic. Alternatively, the animals may be exposed to at least three different concentrations in order to determine the acute toxicity of the substance (concentration at which 50% lethality occurs; LCD50). The present test neither tested a limit concentration nor conducted a selection of concentrations that would allow the LC50 to be determined.

The OECD test guidelines 412 (OECD, 2018a) and 413 (OECD, 2018b) for subacute/subchronic inhalation studies explicitly state that three different test concentrations should be used and that the highest test concentration selected should cause clear toxicity without leading to lethality or suffering of the animals. If the test substance is completely non-toxic, this should be demonstrated by testing a high limit concentration, which should prove the non-toxicity of the substance. However, the authors of the study used only one, relatively low test concentration (no limit concentration). No toxicity was observed at this concentration. The authors of the study do not elaborate on why only such a low concentration of WS-23 was tested. The absence of obvious toxicity after subacute inhalation of WS-23 could – in addition to the low test concentration – also be due to the significantly lower daily exposure duration of the rats compared to the 6h/day specified in recognised test guidelines (day 1 and 2: 60 min; day 3 - 5: 90 min; day 6 - 28: 120 min). In addition, the histopathological analysis was only qualitative and not based on a quantitative assessment. Raw data on the histopathological findings are not available. The lack of histopathological evidence of organ toxicity is in contradiction to previous studies on the oral toxicity of WS-23 in rats, which reported kidney damage and hepatotoxicity from as little as 10 mg/kg bw/day (NOAEL of 5 mg/kg bw/day, based on an EFSA report from 2015, which was based on a 14-week study). Although initial indications of possible kidney damage are not mentioned by the authors of the study (Wu et al. 2021), they are recognisable after detailed examination of the available raw urine test data based on increased protein levels in the urine (significant in females) directly after the 28-day inhalation exposure. Why the relevance of these findings was not clarified by testing higher WS-23 concentrations remains open and calls into question the meaningfulness of this study. The discrepancy in the

severity and nature of the effects between the oral and inhalation studies could also be due to the different routes of exposure (oral vs. inhalation) or the different duration of exposure (14 weeks for oral administration vs. 4 weeks for inhalation).

The NOAEL of 5 mg/kg bw/day used in the EFSA report (2015) is similar to the NOAEL of 10 mg/kg bw/day reported in a toxicological assessment by Api et al. (2024). The EFSA report (2015) describes the results of three studies in Sprague-Dawley rats treated by gavage: A 14-day study with groups of six rats of both sexes receiving twice-daily doses of 0, 5, 25 or 50 mg/kg bw in corn oil; a 14-week study with groups of 30 rats of both sexes receiving once-daily doses of 0, 10, 50 or 100 mg/kg bw in corn oil; and another 14-week study with groups of 30 rats of both sexes receiving once-daily doses of 0, 1, 2, 5, 10 or 50 mg/kg bw in corn oil. The studies showed treatment-related hepatic and renal toxicity at doses of 10 mg/kg bw and higher. The NOAEL was set at 5 mg/kg bw/day based on histopathological changes in the kidneys of male rats in the 14-week study with doses of 0, 1, 2, 5, 10 or 50 mg/kg bw in corn oil. These studies have not been made available to the BfR.

The NOAEL used by Api et al. (2024) was derived from a GLP-compliant 90-day study in which male and female Sprague-Dawley rats were exposed to WS-23 at doses of 10, 50 or 100 mg/kg bw/day in corn oil. The study revealed a significantly increased relative and absolute liver mass in the medium- and high-dose groups after 6 weeks in female animals and after 13 weeks in male and female animals of the high-dose group. Histopathological analyses showed treatment-related hepatic fatty degeneration and renal tubular nephrosis in male rats in the medium- and high-dose groups after 6 weeks. Females showed no hepatic changes but also showed tubular nephrosis in these groups. After 13 weeks, hepatic fatty degeneration was observed in male and female animals in the medium- and high-dose groups. Based on the liver and kidney effects, the NOAEL of this study was set at 10 mg/kg bw/day. However, further studies are needed to assess long-term health hazards and risks, especially after inhalation intake of WS-23.

Potential carcinogenic and mutagenic effects: Currently, there is no clear evidence that WS-23 is carcinogenic or mutagenic. However, *in vitro* genotoxicity studies on mammalian cells have shown that WS-23 is clastogenic, i.e. it can cause disruption or breakage of chromosomes, which led to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) requesting additional toxicological studies to further assess the safety of WS-23. In some of the studies submitted to JECFA, WS-23 caused neither chromosomal aberrations in rat bone marrow cells nor effects in the comet assay in female rat kidney cells. By contrast, it was weakly genotoxic in the comet assay in kidney cells of male rats. It was postulated that this effect was sex-specific for males. The Committee also noted that no data were presented on the potential of this compound to form reactive metabolites and concluded that the previously expressed concerns regarding *in vivo* genotoxicity had not been adequately addressed (FAO & WHO, 2016). This is another reason why some uncertainty remains, especially with regard to chronic inhalation, as no data on these issues are available to date.

Epidemiological and clinical observations: The BfR is not aware of any epidemiological or clinical studies on the above substance.

WS-3

Synthetic coolants such as WS-3 are classified as GRAS (Generally Recognised as Safe) by the Flavour Extracts Manufacturers Association of the United States (FEMA). However, this classification only applies to their intended use in food and not for use in e-cigarettes, where the substance is inhaled. WS-3 is not classified by the REACH registrants of the substance for human health hazard classes according to CLP.

While the toxicological profile of this coolant has been relatively well studied in terms of oral and dermal exposure, there is a lack of inhalation toxicity data required for the assessment of its use in e-cigarettes.

In vitro

No studies have been made available to the BfR.

In vivo

Two studies with WS-3 in rats are mentioned in EFSA (2008, 2010) and FHO/WHO (2016) reports. The original study reports or publications are not available to the BfR. The studies mentioned in these reports can also be found in detail in the ECHA database: In a 28-day study, rats were treated by gavage with WS-3 (doses of 0, 8, 40, 200 and 1000 mg /kg bw/day (Miyata, 1995). Doses ≥ 40 mg/kg bw/day resulted in damage to the liver, spleen and kidneys. A NOAEL of 8 mg/kg bw/day was determined based on this 28-day study in rats. This value is also given in various databases, such as the QSAR Toolbox and the EFSA Chemical Hazards Database: OpenFoodTox 2.0, and serves as a reference point for the assessment of the safety of WS-3 in toxicological studies. In a 22-week study by the same author (Miyata, 1995), rats were treated with WS-3 by gavage at the following doses: 0, 100, 300 and 725 mg WS-3/kg bw/day. In this study, even at the lowest daily dose of 100 mg/kg, mild toxicity was observed in the liver and kidneys (EFSA, 2008; ECHA). A NOAEL could not be determined in this study, the LOAEL (Lowest Observed Adverse Effect Level) is therefore 100 mg/kg bw/day.

Further oral studies with repeated dosing in dogs (beagle) resulted in LOAELs that were higher than those in the rat studies (EFSA, 2008).

WS-5

WS-5 is not classified for human health hazard classes (according to CLP) by the REACH registrants of the substance.

The toxicological data on this substance, especially with regard to inhalation, are very limited.

In vitro

No studies have been made available to the BfR.

In vivo

Oldham et al (2023) conducted a 90-day nasal exposure study in Sprague-Dawley rats on behalf of an e-cigarette manufacturer. WS-5 concentrations of 0.2 wt%, 0.4 wt% and 0.8 wt% were used. The authors reported that no changes in blood serum chemistry, haematology, coagulation, urine, lavage fluid or clinical chemistry were observed at any of the concentrations tested. Macroscopic examinations and organ weights also showed no abnormalities associated with exposure to WS-5. Only a reduction in respiratory rate in the

presence of WS-5 was observed. The authors concluded that based on the absence of adverse effects, the No Observed Adverse Effect Concentration (NOAEC) is 2.5 mg/L aerosolised WS-5 at a concentration of 0.8% by weight, which corresponds to a measured mean exposure concentration of approximately 18 µg WS-5/L. However, it should be taken into account that this study was underdosed, as the highest concentration tested did not cause a clear level of toxicity (without causing lethality or animal suffering) as specified in the validated OECD Test Guideline 412 (OECD, 2018a) for subacute inhalation studies. The publication does not provide a rationale for why only such low test concentrations were used, which calls into question the relevance of this study.

The EFSA report of 2012 (EFSA, 2012b) reported on a 90-day toxicity study in rats in which the substance was administered orally by gavage. The study was conducted in accordance with OECD guideline TG 408 under GLP conditions. Doses of 0, 25, 75, 225 and 675 mg WS-5/kg bw per day were administered to 10 animals per sex and dose group. Recovery groups were included in the control and maximum dose groups. Test substance-related microscopic findings were observed in the kidneys (tubular degeneration and dilatation, interstitial fibrosis and vacuolisation of the tubular epithelium) and in the liver (periportal hepatocyte vacuolisation and centrilobular hepatocyte hypertrophy) in male and female rats. In the female animals, an increased incidence of cardiomyopathies was also observed at a dose of 675 mg/kg bw/day. Microscopic changes in the kidneys, liver and heart were also present after the recovery phase, albeit with less severity. Renal damage in male and female rats and cardiomyopathies in female rats at the dose of 675 mg/kg bw/day were assessed as adverse effects. In addition, haematological changes were noted at doses of 225 and 675 mg/kg bw/day, which were also considered to be adverse, as their occurrence was dose-dependent. Changes in red blood cells were present in the highest dose group even after the 14-day recovery period. Therefore, the Committee established a NOAEL of 75 mg/kg bw/day.

3.1.3 Exposure assessment

Intake routes and exposure scenarios: The primary route of exposure for WS-23, WS-3 and WS-5 associated with e-cigarettes is inhalation. The substances are dissolved in the e-liquids and converted into an aerosol by vaporisation in the e-cigarette, which enters the respiratory tract directly. The inhalation exposure depends on several factors, including the concentration of the cooling substances in the e-liquid, the user's consumption behaviour, the efficiency of the vaporisation device and the resulting amount of aerosol inhaled per puff. In addition to inhalation, dermal exposure, e.g. through skin contact with spilled e-liquid, and to a lesser extent oral exposure, e.g. through swallowing small amounts of the liquid, can also occur. However, these pathways are considered to be less relevant when considering e-cigarettes compared to inhalation intake. Other potential sources of exposure, such as from food and cosmetics, were not considered in this risk assessment. However, these might additionally increase the overall exposure to coolants.

WS-23

A search of the EU-CEG database carried out by the Ministry of Agriculture and Consumer Protection of North Rhine-Westphalia revealed that of the total of around 426,350 registered products, over 78,000 contain WS-23. Of these, 43% have a WS-23 content of less than 1.0% by weight, 49% of the products have a concentration of between 1.0 and 4.0% by weight, and 8% of the products contain more than 4.0% by weight. Furthermore, the State

Agency for Nature, Environment and Consumer Protection of North Rhine-Westphalia (LANUV) carried out an exposure assessment of WS-23 based on the importer's stated concentrations of 4.125% by weight in the disposable e-cigarettes concerned. The affected products are disposable e-cigarettes

In a Maryland study with 91 participants, the average consumption of e-liquid was 6.3 ml/day with a variation between 0.14 and 31 ml/day (Tillery et al., 2023). Another study involving 309 people in Greece, 82.5% of whom were daily e-cigarette users, found an average liquid consumption of 5.1 ml/day (Diamantopoulou et al., 2019). In a study in Maryland with 100 participants who used e-cigarettes daily and exclusively, consumption ranged from 5 to 240 ml e-liquid/week, with an average consumption per day of 7.6 ml (Aherrera et al., 2020). Another study conducted in Germany on the consumption behaviour of adolescents and young adults showed an average e-liquid consumption of 2.1 ml/day with a maximum consumption of 12 ml/day when using disposable e-cigarettes. With rechargeable devices, an average of 4.6 ml was consumed daily, with a maximum consumption of 40 ml/day (Gali et al., 2022).

Based on the studies mentioned above, three scenarios for the daily consumption of e-liquid were considered: low, medium and maximum with values of 1, 5 and 40 ml/day, respectively. Assuming a concentration of 4% by weight of WS-23 in e-liquid (based on EU CEG data) and a density of 1.1105 g/ml, the daily intake is approximately 44 mg WS-23 for a consumption of 1 ml of e-liquid, 222 mg WS-23 for 5 ml of e-liquid and 1,776 mg WS-23 for 40 ml of e-liquid. The calculations thus cover a range from low to high consumption assumptions. The density of 1.1105 g/ml was previously determined by the Chemical and Veterinary Investigation Office (Chemisches und Veterinäruntersuchungsamt) in Ostwestfalen-Lippe (CVUA OWL) for a comparable e-Liquid.

WS-3

Based on samples tested by the Sigmaringen Chemical and Veterinary Investigation Office (Chemisches und Veterinäruntersuchungsamt), concentrations of WS-3 of up to 8.3 mg/ml were found. According to the EU CEG database, WS-3 was found in 20,619 registered products, with 61% of these products having concentrations between 1 and 10 mg/ml and 18% having concentrations between 10.1 and 50 mg/ml. Based on these data, a representative concentration of 4% by weight of WS-3 in the e-liquid was determined for further assessment. Taking into account a density of 1.1105 g/ml and three different consumption quantities, this results in a daily intake of approximately 44 mg WS-3 for a consumption of 1 ml, 222 mg for 5 ml and 1,776 mg for 40 ml of e-liquid.

WS-5

The BfR has no official measurement data on the concentration of WS-5 in the various e-liquids. However, a search of the EU-CEG database carried out by the North Rhine-Westphalia State Office for Nature, Environment and Consumer Protection identified concentrations of 1 to 4% by weight. Based on this data, a concentration of 4% by weight was used for the exposure assessment. Based on these data and taking into account a density of 1.1105 g/ml and a daily consumption of 1 ml (low), 5 ml (medium) or 40 ml (maximum), this results in an intake of about 44 mg WS-5 for a consumption of 1 ml, 222 mg for 5 ml and 1,776 mg for 40 ml of e-liquid. However, it must be taken into account that the

highest percentage given (4% by weight) was used to reflect the maximum exposure of consumers (worst-case assumption).

3.1.4 Risk characterisation

WS-23

After oral subchronic exposure, the observed treatment-related hepatic and renal toxicity at ≥ 10 mg/kg bw/day resulted in a NOAEL of 5 mg/kg bw/day for WS-23 (see 3.1.2). From the available data of a subacute inhalation toxicity study with WS-23 in Sprague-Dawley rats (Wu et al. 2021), an inhalation NOAEC of 342 mg/m³ (29 mg/kg bw/day) was determined for WS-23. Nevertheless, these inhalation data are not used as the basis for an assessment due to the poor quality of the study.

The LANUV used a reproducible approach with a margin of exposure (MOE) of 100 for its assessment of WS-23 in e-cigarettes. In the present risk assessment for WS-23, a safety margin of 200 was used to take into account the uncertainty factor of 2 for the transfer of results from a subchronic study to chronic exposure and thus better cover long-term effects (EFSA, 2012a).

Based on the mean daily consumption of 5 ml of e-liquid (see 3.1.3) and the NOAEL of 5 mg/kg bw/day, a risk to consumer health was determined as the resulting MOE values are below the safety margin of 200. Due to the very different individual daily e-liquid consumption, MOE calculations were carried out with three different consumption levels (see Table 1). In all calculated scenarios, a health risk was determined using the MOE approach.

As an alternative approach, the DNEL was calculated and compared with the estimated exposure. The DNEL for inhalation exposure to WS-23 is calculated here taking into account relevant uncertainty factors in accordance with the REACH guidelines, based on the subchronic NOAEL determined for rats (5 mg/kg bw/day). In order to calculate the DNEL for inhalation exposure to WS-23 in accordance with REACH guidelines, the NOAEL determined in the oral study must first be converted into an inhalation NOAEC (no observed adverse effect concentration). A respiratory volume rate of the rat of 1.15 m³/kg bw per day was used for 24-hour exposure (ECHA, 2012). It was assumed that 100% of the substance absorbed by inhalation is available systemically, as is also assumed after oral application. This results in a NOAEC of 4.35 mg/m³. To derive a chronic DNEL for the general population, the NOAEC from the above-mentioned animal study was divided by a cumulative uncertainty factor (AF) of 100, which (according to REACH guidelines) takes into account the following aspects: inter-specific differences between humans and rats with an overall factor of 2.5 (for toxicodynamic differences), inter-individual variability with an AF of 10, extrapolation from subchronic to chronic exposure with an AF of 2. A further AF of 2 was added for the quality of the database, as although the NOAEL was selected on the basis of a reliable report by the "Joint FAO/WHO Expert Committee on Food Additives", the report only provides very rough information on the study design, the validity of the study and the effects found (only: "histopathological lesions of the kidney"). The study report is not available.

Taking into account the cumulative uncertainty factor of 100, this results in a DNEL of 0.04 mg/m³ (Table 1).

Table 1: MOEs and RCRs for WS-23 under different exposure scenarios.

NOAEL (mg/kg bw/d)	E-liquid consumption (ml/day)	WS-23 intake (mg/d)*	WS-23 intake (mg/m³/d)**	NOAEC, extrapolated (mg/m³)	MOE	DNEL (mg/m³)_long-term, inhalation, general population, 24 h/d	RCR (exposure/ DNEL)
5	1	44.42	2.22	4.35	**1.96**	0.04	**55.5**
	5	222.10	11.11		**0.39**		**277.8**
	40	1776.80	88.84		**0.05**		**2221.0**

*Under the assumption of 4% by weight of WS-23, a density of 1.1105 g/ml and ** a breathing volume of 20 m³/person for 24 h.

For the assessment of the substance WS-23, it has been shown that both the MOE approach and the DNEL method lead to comparable results, especially with regard to the risk potential of WS-23. This underlines the consistency of the two approaches and provides additional certainty in the toxicological assessment. The risk characterisation shows that the current estimated exposure to WS-23, especially with intensive use of e-cigarettes, could pose a significant health risk with regard to systemic effects on the liver and kidneys: At the assumed different e-liquid consumption levels (1, 5 and 40 ml per day), all calculated MOE values (between 0.05 and 1.96) fall well below the safety margin of 200 (including extrapolation from subchronic to chronic exposure with an AF of 2). This, as well as the exceeding of the RCR of 1, indicates that a hazard to health (liver and kidney effects), including chronic effects, cannot be excluded. No statement can be made regarding harmful effects in the lungs after inhalation intake due to a lack of robust data.

WS-3

A NOAEL of 8 mg/kg bw/day was determined for WS-3 in a 28-day study in rats (see 3.1.2). The intake of WS-3 calculated in Table 2 takes into account different amounts of e-liquid consumption (see 3.1.3). In the present risk assessment for WS-3, a safety margin of 600 was used to take into account the uncertainty factor of 6 for the transfer of results from a subacute study to chronic exposure and thus better cover long-term effects (EFSA, 2012a). Taking these factors into account, the MOEs determined for all exposure estimates considered, with values between 0.08 and 3, are well below the applied safety margin of 600 (Table 2), indicating a risk to consumer health.

As an alternative approach, the DNEL was also calculated here and compared with the exposure determined. In order to calculate the DNEL for the inhalation exposure of WS-3 in accordance with the REACH Directive, the NOAEL determined in the oral subacute study (8 mg/kg bw/day) must first be converted into a NOAEC (see WS-23). Here, too, it was assumed that 100% of the substance absorbed by inhalation is completely available systemically, as is also assumed after oral application. This results in a NOAEC of 6.96 mg/m³. To derive the DNEL, the NOAEC was divided by a cumulative uncertainty factor (AF) of 150, which takes into account the following aspects according to REACH guidelines: interspecies differences between humans and rats with an overall factor of 2.5 (for toxicodynamic differences), interindividual variability with an AF of 10, extrapolation from subacute (28 days) to chronic exposure with an AF of 6. For the quality of the database, no further AF was added, as the NOAEL is based on reliable information from the ECHA database. However, the study report is not available. The calculations resulted in a DNEL of 0.05 mg/m³.

Table 2: MOE and RCR for WS-3 for different exposure scenarios.

NOAEL (mg/kg bw/d)	E-liquid consumption (ml/d)	WS-3 intake (mg/d)*	WS-3 intake (mg/m³/d) **	NOAEC (mg/m³)	MOE	DNEL (mg/m³)_long-term, inhalation, general population, 24 h/d	RCR (exposure/ DNEL
8	1	44.42	2.22	6.96	**3.14**	0.05	**44.4**
	5	222.10	11.11		**0.63**		**222.2**
	40	1776.80	88.84		**0.08**		**1776.8**

*Under the assumption of 4% by weight of WS-3, a density of 1.1105 g/ml and ** a breathing volume of 20 m^3/person for 24 h.

For the assessment of WS-3, the MOE approach and the DNEL method show comparable results, which confirms the consistency of the toxicological assessment. For the assumed different e-liquid consumption levels (1, 5 and 40 ml per day), all calculated MOE values fall well below the recommended safety margin of 600 (including extrapolation from subacute to chronic exposure with an AF of 6). This indicates an increased health risk when using the substance in e-liquids. Similarly, the calculated RCRs of > 1 indicate that the use of WS-3 in e-liquids is associated with an increased health risk (liver and kidney effects). It should also be noted here that due to the limited data available, no statements can be made on local effects in the lungs after inhalation of the substance.

WS-5

For the calculation of the MOE for WS-5, a NOAEL of 75 mg/kg bw/day and a WS-5 fraction of 4% by weight were used (see 3.1.2 and 3.1.3).

Exposure was again extrapolated for three consumption levels, with MOE values consistently below the established safety margin of 200, indicating that a health risk cannot be ruled out (Table 3).

As an alternative approach, the DNEL was also calculated here and compared with the exposure determined. The DNEL for the inhalation exposure of WS-5 is calculated here taking into account relevant uncertainty factors in accordance with the REACH guidelines, based on the NOAEL determined for rats (75 mg/kg bw/day). In order to calculate the DNEL for the inhalation exposure of WS-5 according to REACH guidelines, the NOAEL determined in the oral study must first be converted into a NOAEC (see above). Here, too, it was assumed that 100% of the substance absorbed by inhalation is available systemically, as is also assumed after oral application. This results in a NOAEC of 65.22 mg/m³. To derive the DNEL, the NOAEC was divided by a cumulative uncertainty factor (AF) of 100, which takes into account the following aspects: interspecies differences between humans and rats with an overall factor of 2.5 (for toxicodynamic differences), interindividual variability with an AF of 10, extrapolation from subchronic to chronic exposure with an AF of 2. For the quality of the database, a further AF of 2 was added. The study report is not available.

Taking into account the cumulative uncertainty factor of 100, this results in a DNEL of 0.65 mg/m³ (Table 3).

Table 3: MOE and RCR for WS-5 for different exposure scenarios.

NOAEL (mg/kg bw/d)	E-liquid consumption (ml/d)	WS-5 intake (mg/d)*	WS-5 intake (mg/m³/d)**	NOAEC (mg/m³)	MOE	DNEL (mg/m³)_ long-term, inhalation, general population, 24 h/d	RCR (exposure/ DNEL
	1	44.42	2.22		**29.38**		**3.4**

75	5	222.10	11.11	**65.22**	**5.87**	**0.65**	**17.1**
	40	1776.80	88.84		**0.73**		**136.7**

*Under the assumption of 4% by weight of WS-5, a density of 1.1105 g/ml and ** a breathing volume of 20 m^3/person for 24 h.

For the assessment of WS-5, the MOE approach and the DNEL method show comparable results, which underlines the consistency and reliability of the toxicological assessment. The risk characterisation shows that all calculated MOEs are below the value of 200. This indicates an insufficient margin of safety, especially at higher consumption levels, suggesting that a health risk cannot be excluded. Furthermore, the RCRs clearly exceed the value of 1, indicating that systemic toxic effects, such as renal effects and cardiomyopathy, including chronic effects, cannot be excluded for all exposure scenarios determined. It should again be noted that due to a lack of data, no statements can be made on local effects in the lungs after inhalation of the substance.

3.2 Risk management options, recommended measures

Establishment of exposure limits and quantity restrictions: In view of the potential health risks identified, particularly with regard to the systemic toxicity (damage to the liver and kidneys) that can be caused by the substances, but especially with regard to the very low MOEs and very high RCRs for WS-23, WS-3 and WS-5, the use of these coolants in e-liquids is not recommended. Other possible sources of exposure, such as from food and cosmetics, were not taken into account in this risk assessment, though these might have an additional impact on the MOE or RCR.

Furthermore, the genotoxicity, in particular the clastogenic effect, of WS-3, WS-5 and WS-23 should be further investigated to allow for the establishment of a complete risk profile.

3.3 Other aspects

Current research provides clear evidence that the use of synthetic coolants such as WS-23 in e-cigarettes can substantially influence consumer behaviour and the attractiveness of these products. For example, Tackett et al. (2023) showed in a clinical study that the addition of WS-23 to e-cigarette liquids significantly increases user satisfaction and acceptance. In particular, the products with WS-23 were perceived as more pleasant, colder, and less harsh, which led to an increased willingness to use these products again, regardless of the basic flavour, nicotine concentration or tobacco consumption status of the participants. These findings suggest that WS-23 plays a central role in making the vaping experience more appealing and potentially lowering the inhibition threshold for consumption, especially among inexperienced or young users. In addition to refinement of the sensory experience, a targeted search of PubMed and other bibliographic sources by Talhout et al. (2023) shows that coolants such as WS-23 can reduce the irritation caused by nicotine and other components of the aerosol by activating TRPM8 receptors. This reduction in sensory acuity not only promotes product acceptability, but could also change inhalation behaviour by facilitating deeper, faster and larger puffs. These behavioural changes could lead to an increased nicotine intake and an associated stronger dependence.

The studies mentioned above underline that synthetic coolants such as WS-23 not only increase the attractiveness of e-cigarettes, but can also influence inhalation behaviour and the associated nicotine intake. The potential effect on young and inexperienced users, for

whom the inhibition threshold for starting to use nicotine products could be lowered, is of particular concern.

The fact that these synthetic cooling agents are used in products to potentially circumvent regulatory restrictions on menthol in conventional tobacco products and novel tobacco products is of further relevance. As these substances are odourless, they do not fall under the definition of "characterising flavourings", making them an effective method of circumventing existing and future regulations. In addition, another study from the USA by Jabba et al. (2023) shows that synthetic coolants are also used in other nicotine-containing products, such as oral nicotine pouches, to increase the attractiveness of these products.

Further information on e-cigarettes on the BfR website

Questions and answers on e-cigarettes – anything but harmless
https://www.bfr.bund.de/cm/349/frequently-asked-questions-about-e-cigarettes.pdf

The BfR science comic „What does science say?“
https://www.bfr.bund.de/cm/349/what-does-science-say-the-bfr-science-comic-e-cigarettes.pdf

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The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. The BfR advises the Federal Government and the States ('Laender') on questions of food, chemicals and product safety. The BfR conducts independent research on topics that are closely linked to its assessment tasks.

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