Scientific Committee on Consumer Safety

SCCS

OPINION ON

the safety of the use of formaldehyde in nail hardeners

The SCCS adopted this opinion by written procedure

on 7 November 2014

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

The substance formaldehyde (CAS Number 50-00-0) is anticipated to be classified as a carcinogen category 1B under the CLP Regulation (EC) No 1272/2008, following a qualified majority in favour of the Committee opinion adopted on 17 December 2013. This classification shall apply by 1st April 2015.

Regulation (EC) No 1223/2009 foresees that the use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR) substances, of category 1A or 1B, under Part 3 of Annex VI to the Regulation (EC) No 1272/2008, is prohibited.

However, such substances may be used in cosmetic products by way of exception where, subsequent to their classification as CMR substances of category 1A or 1B under Part 3 of Annex VI to Regulation (EC) No 1272/2008, all of the conditions (hereafter reported) of Article 15.2 of the Cosmetics Regulation are fulfilled:

(a) they comply with the food safety requirements as defined in Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matter of food safety;

(b) there are no suitable alternative substances available, as documented in an analysis of alternatives;

(c) the application is made for a particular use of the product category with a known exposure; and

(d) they have been evaluated and found safe by the SCCS for use in cosmetic products, in particular in view of exposure to these products and taking into consideration the overall exposure from other sources, taking particular account of vulnerable population subgroups.

The regulatory measures authorising such exemption for use shall be adopted within 15 months of the classification as CMR 1A or 1B of the substance(s) concerned in Part 3 of Annex VI to Regulation (EC) No 1272/2008.

Nail hardeners are very specialized cosmetics used to harden or strengthen natural nails, especially soft, brittle or fragile nails. Formaldehyde is used in nail hardeners for its specific cross-linking functionality with keratin. The use of formaldehyde in nail hardeners is currently restricted as specified in the Entry 13 of Annex III of Regulation (EC) No 1223/2009 – i.e., a maximum concentration in the finished products of 5% (as formaldehyde); labelled as *'contains formaldehyde'* when the finished cosmetic product contains formaldehyde in a concentration above 0.05% and with the warning *'protect cuticules with grease or oil'*.

Additionally the use of formaldehyde (CAS 50-00-0) as preservative for cosmetics is also allowed under restricted conditions [Entry 5 of Annex V].

In July and November 2013, Cosmetics Europe representing an industry consortium, submitted a full application to support the use of formaldehyde in nail hardeners at the maximum level of 2.2% (as free formaldehyde*).

The industry consortium considers that its application for exemption contains the data supporting the fulfilment of conditions a), b) and c) of Article 15.2. The application contains an evaluation of the toxicological profile of formaldehyde, a global human exposure

assessment to formaldehyde and an assessment of the safety of free formaldehyde* released by the usage of nail hardeners under the current conditions for use (see above).

The Commission published on 23 May 2013, a call for data on formaldehyde use in cosmetics and/or formaldehyde released by others substances used in cosmetics, seeking also information of the suitable alternatives. The Commission only received information from Cosmetics Europe that is included in the application submitted.

The Commission considered that the substantiation of the three first conditions of Article 15.2 are fulfilled, allowing that the SCCS proceeds now with its scientific assessment.

2. TERMS OF REFERENCE

In view of above, and taken into account the data provided in the application, and from other sources, SCCS is requested:

1) To assess if condition d) of Article 15.2 is fulfilled, in order to confirm or not the safe use of formaldehyde in nail hardeners at the maximum level of 2.2% (as free formaldehyde).

2) To indicate if there are any further scientific concerns with regard to the use of formaldehyde in nail hardeners.

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Formaldehyde/Formaldehyde

3.1.1.2 Chemical names

Methanal (IUPAC), Formalin, Methyl aldehyde

3.1.1.3 Trade names and abbreviations

Formol, Fannoform, Lysoform, Morbicid acid, Superlysoform

3.1.1.4 CAS / EC number

CAS: 50-00-0
EC: 200-001-8

3.1.1.5 Structural formula

$H_2C=O$

3.1.1.6 Empirical formula

CH_2O

3.1.2 Physical form

Gaseous

3.1.3 Molecular weight

Molecular weight: 30.0258 g/mol

3.1.4 Purity, composition and substance codes

100% (≤55% in aqueous solution)

3.1.5 Impurities / accompanying contaminants

Methanol (CAS 67-56-1): 0.5% – 2% (starting material)
Methanol (CAS 67-56-1): <16% (stabilizer)
Formic acid (CAS 64-18-6): ~ 0.3% (manufacturing process)
Isophthalobis guanamine (CAS 5118-80-9): 0.1% — 0.5% (stabilizer)
Water: >45% (additive, solvent)

3.1.6 Solubility

≤55% [in water at room temperature, CH_2O becomes converted into methylene glycol ($HOCH_2OH$ = formaldehyde hydrate), and further polymerized into oligo- and poly(oxy-methylene)glycols = paraformaldehyde]

Formaldehyde is soluble in ethanol, diethylether, toluene, chloroform, ethylacetate, etc.

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: 0.35 at 25°C

3.1.8 Additional physical and chemical specifications

Melting point:	—92°C
Boiling point:	—19.1°C
Flash point:	85°C (37% aqueous solution, methanol-free)
	50°C (15% aqueous solution, methanol-free)
Vapour pressure:	5185 hPa or 3890 mm Hg at 25°C
Density:	1.067 (in air, air = 1)
	0.815 g/cm^3 (water, at —20°C)
Viscosity:	2.58 centi poise at 25°C
pKa:	13.3
Refractive index:	1.3746 (at 20°C, 37% aqueous solution, methanol-free)
pH:	2.8 – 4.0 (37% aqueous solution, methanol-free)
UV_Vis spectrum (….. nm):	328, 340, 354 nm
Conversion ppm – mg/m^3:	1 ppm = 1.228 mg/m^3 (25°C / 1 atm)
	0.0815 ppm = 100 µg/m^3 (25°C / 1 atm)

3.1.9 Homogeneity and Stability

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General comment to physico-chemical characterisation

Formaldehyde is a gas with a pungent suffocating odor that is stable under usual pressure and temperature conditions. At concentrations >30% formaldehyde becomes oligo- and polymerised (cloudy) in aqueous solutions unless methanol is present as polymerisation inhibitor.

3.2. Function and uses

The use of formaldehyde as preservative for cosmetics is allowed under restricted conditions (Annex V of Regulation (EC) No 1223/2009). In addition, formaldehyde is added in nail hardeners for its specific cross-linking functionality with keratin (restriction according to Entry 13 of Annex III). The use of formaldehyde in cosmetics now requires reevaluation due to its classification as carcinogen category 1B under CLP Regulation. In this opinion only the safety of formaldehyde in nail hardeners with up to a maximum concentration of 2.2% is being considered and evaluated.

Nail hardeners are very specialised cosmetics used to harden or strengthen natural nails, especially soft, brittle or fragile nails. Less than 1% of the entire category of nail polishes and nail treatments are nail hardeners. Industry claims that formaldehyde, due to its cross-linking functionality and its low molecular weight, is the only substance which can effectively harden the nail plate. Therefore most nail hardeners use formaldehyde dissolved in water (i.e., formaldehyde hydrate = methylene glycol = “formalin”) as the nail hardening ingredient. The principal mode of action of formaldehyde in terms of hardening and strengthening the nail is the cross-linking of keratin in the nail plate. To achieve the hardening effect, the nail hardener is brushed onto the whole or certain parts (tip, half) of the nail plate.
Nail hardeners are supposedly not used on a daily basis (exact frequency of usage remains unclear). Due to the different composition of the commercial products, the instructions of the manufacturers vary in their recommendation for the frequency of usage (estimates range between about 65 times/yr up to 124 times/yr are possible). Considering the treatment area of each of the 10 fingernails, the total amount of nail hardeners needed lies in the range of 14 – 250 mg/treatment (finger nail tips up to complete nail plate corresponding to about 4 – 11 cm² according to SCCS Notes of Guidance, 2012). Currently there is no data available on the frequency of use of nail hardeners to treat toenails.

The following use of nail hardeners has been stated in different product descriptions (according to Andersen *et al.*, 2008, Danish EPA document, Chapter 10, Annex):

1. Apply one layer on the first day and a second layer on the second day.
2. On the third day remove both layers and start again.
3. Repeat these steps for 14 days.
4. After this 14-day procedure the product should only be used 1-2 times per week.
5. The 14-day treatment should not be repeated more than 1-2 times a year.

Estimating a life-time exposure based on these instructions will result in the following maximum use: 2 times x 14 days = 28 times; rest of the year 2 times per week (48 weeks x 2 times) = 96 times. The maximum use will be 124 times/year.

3.3. Toxicological Evaluation

The toxicological profile of formaldehyde is exhaustively described in the literature. An extended summary is given in the Annex to this opinion. See the Annex for acute toxicity, subchronic toxicity, reproductive toxicity, mutagenicity, carcinogenicity, skin and mucous membrane irritation, sensitisation, dermal absorption and toxicokinetics of formaldehyde.

In light of the data compiled in the Annex of this opinion, the SCCS concludes as follows:

Toxicokinetics:
Formaldehyde is an intermediate in the physiological carbon-1 pool and present in measurable concentrations in all metabolically active cells and tissues. If inhaled,

formaldehyde can react directly with mucus or with macromolecular cellular components at the site of first contact. No change in urine concentrations of its oxidation product, i.e., formic acid, were observed in human volunteers at air concentrations <0.5 ppm (for 3 weeks). Similarly, no significant changes in the blood concentration of formaldehyde were found after inhalation of 1.9 ppm by six human volunteers (40 min), at 14.4 ppm in rats (2 h), and at 6 ppm in monkeys (6 h/day, 5 days/wk, 4 weeks). Still, animal studies clearly demonstrate penetration of radioactively labelled formaldehyde through the skin (0.5 – 9%, depending on the species investigated).

Sensitisation:
The strong skin sensitising properties of formaldehyde have been proven in numerous animal studies. In the LLNA in mice, an EC_3 value of 0.29% has been established. In humans, formaldehyde induced contact dermatitis in a concentration- and patch test condition-dependent manner. In the human repeat insult patch test, positive responses started at 1% (4.5% of the patients positive). Based on this a threshold value for the elicitation of sensitised individuals has been suggested at 30 ppm (0.003%; aqueous solution) and 60 ppm (0.006%; products containing formaldehyde), respectively. In the past, several multicentre studies in Europe showed a prevalence of allergy against formaldehyde in clinical patients in the range of 0.7 – 3.6%. This rate in patients was shown to remain stable over the last several years. In the general population the prevalence of contact allergic reactions to formaldehyde is estimated to be below 0.5%.
On the contrary, there is currently no experimental evidence based on animal studies that formaldehyde might be similarly capable to trigger sensitisation in the respiratory tract. Although one human study demonstrated a decrease of >15% in the peak expiratory flow rate after acute challenge with 2 ppm formaldehyde (12 out of 230 patients occupationally exposed to formaldehyde), the symptoms observed might be rather due to the fact that it is an irritant.

Irritation:
Formaldehyde is a known skin irritant and exposure as a vapour also results in irritation of eyes, nose and throat. Odor detection occurs well below 1 ppm in most humans. In a group of 50 subjects, the 50-percentile detection threshold was 0.145 ppm, the 10-percentile detection threshold 0.020 ppm and the 90-percentile threshold 0.5 ppm (WHO, 1989). Eye irritation was revealed as most sensitive adverse endpoint. In susceptible individuals, slight discomfort due to eye irritation occurred at 0.25 ppm but dose-dependent increases in eye irritation were not observed below 1 ppm. No subjective symptoms were noted at 0.15 ppm. By contrast, nose and throat irritations occur at dose levels of ≥2 ppm. Objective ratings for eye irritation (conjunctival redness and eye blinking frequency) have been investigated in healthy volunteers and a NOAEL of 0.5 ppm (without exposure peaks) and 0.3 ppm (with exposure peaks of 0.6 ppm) was established.

Repeated dose toxicity:
In repeated oral dose toxicity studies, based on local lesions in the stomach of rats, a NOAEL was established at 10 mg/kg bw/day. In the skin, irritating effects were observed at ≥0.5% formaldehyde. Currently there is no evidence that systemic effects would occur after repeated dermal application of this compound. In rodents and monkeys the respiratory epithelium in the nasal cavity was shown to be the most sensitive site when formaldehyde is being administered via inhalation (induction of squamous metaplasia and hyperplasia). The lowest NOAEL observed in one of the published studies was 0.3 ppm in rats and 0.2 ppm in monkeys.

Mutagenicity and genotoxicity:
Formaldehyde has been demonstrated as being genotoxic and mutagenic *in vitro* as well as *in vivo* at local sites of exposure, both in animals and humans. Oral studies in experimental animals at high doses did not show systemic genotoxicity or mutagenicity. For the inhalation route, however, the situation is less clear: whereas the majority of studies with rats and monkeys were negative, there is currently uncertainty about whether or not formaldehyde

can express its genotoxicity systemically in mice under certain circumstances.

Carcinogenicity:
Based on the observed non-neoplastic lesions in the stomach of rats, a NOAEL of 15 mg/kg bw/day in males and 21 mg/kg bw/day in females has been derived. The overall conclusion from animal studies point to a low potential for toxicity and to insufficient evidence for local and systemic carcinogenicity of formaldehyde exerted via long-term oral exposure. In terms of dermal exposure, some specialised animal studies demonstrate local irritation, but no tumourigenicity in skin. Although formaldehyde has significantly reduced the latency time until the onset of tumours in an initiation/promotion experiment, overall there is no evidence supporting any carcinogenic effect of formaldehyde itself when applied dermally.
By contrast, the inhalation carcinogenicity of formaldehyde is well established in rats with induction of tumours at the site of contact. Conversely, there is only limited evidence that carcinogenic effects may occur at distant sites (cf. below) or via other routes of exposure than inhalation. Following inhalation formaldehyde is absorbed and deposited at the site of first contact, whereas systemic blood levels of formaldehyde in animals or humans always remained unaffected (cf. above). In all of the studies conducted, local tumours in the nasal cavity were only observed at doses producing chronic irritation with accompanying inflammation, hyperplasia and metaplasia. Here, the damaged sites in the nasal epithelium corresponded to sites of tumour induction. Literature data provide convincing support for the assumption that regenerative cell proliferation (starting at about 6 ppm) secondary to cytotoxicity correlates with tumour incidence. Based on the observation of hyperplastic and metaplastic responses it thus seems justified to conclude that regenerative proliferation and accompanying inflammation contributes to the effects of formaldehyde. Furthermore, in light of the experimental and mechanistic data, a threshold-type dose-response for the induction of nasal tumours, with regenerative cell proliferation being the trigger in this carcinogenic process, can be assumed.
Consistent epidemiological evidence strongly supports that formaldehyde is also able to induce nasopharyngeal carcinomas in humans. Meta-analyses revealed an increased risk for adenocarcinoma and some evidence for squamous-cell carcinoma associated with high exposure (>1 ppm) to formaldehyde in both men (OR: 3.0; 95% CI: 1.5 – 5.7) and women (OR: 6.3; 95% CI: 2.0 – 19.7). Conversely, there is only weak epidemiological evidence for a causal relationship between formaldehyde exposure and the induction of myeloid leukaemia. Given the contrasting results on systemic genotoxicity of formaldehyde found in animals (cf. above) however, it cannot be excluded that – under certain circumstances – formaldehyde might also trigger the onset of leukaemia.

Reproductive and developmental toxicity:
There is currently no evidence for reproductive or developmental toxicity of formaldehyde in humans when exposed via inhalation.

3.4. Routes of Exposure

The SCCS evaluation of possible risks associated to formaldehyde exposure from nail hardeners is described in the following sections. As part of this assessment, inhalative exposure to formaldehyde originating from nail hardeners is being discussed in light of aggregate inhalative exposure. The latter results from all other sources of formaldehyde (incl. other cosmetics) that together would contribute to the ambient background levels of this compound.
According to EFSA, background levels of formaldehyde present in foodstuffs are very variable and range from values around 0.1 – 0.3 mg/kg in milk to >200 mg/kg in some fish species (EFSA J, 2014). Considering such wide variability in formaldehyde concentrations in foodstuffs, it was suggested that oral exposure to formaldehyde in humans would not exceed 100 mg formaldehyde per day, corresponding to 1.4 – 1.7 mg/kg bw per day. Unlike the inhalative and dermal exposure routes, gastrointestinal exposure, ingested via

formaldehyde-containing food or water, is not taken into account in the framework of this risk assessment related to the use of formaldehyde-containing nail hardeners.

3.4.1. Inhalation as most relevant route of exposure to formaldehyde that potentially may lead to the induction of cancer

The concentration of endogenous formaldehyde in human blood is about about 2 – 3 µg/ml (2 – 3 ppm, ~0.1 mM); similar concentrations were determined in monkeys and rats. Model calculations considering its half-life and wide distribution in the body and blood estimated that the total body level of formaldehyde at any time is approximately 122.5 mg with an average tissue level of 1.75 mg/kg bw. A half-life of 1.5 min means that half of the 122.5 mg will be used up by either being transferred to the C1 pool or excreted as CO_2 and that an equal amount of formaldehyde will be formed by the organism to maintain the steady state in the blood. This suggests that the human organism produces 2,450 mg per hour or 58,000 mg per day of formaldehyde (Cascieri *et al.*, 1992). Owen *et al.* (1990) calculated that an adult human liver can convert 22 mg formaldehyde per min (1320 mg/h) directly to CO_2.

Exposure to formaldehyde by inhalation has not been found to alter these blood or systemic tissue levels (cf. section 3.3.9.). It has been shown that the primary tissue of contact is usually a very efficient barrier detaining formaldehyde to pass over into systemic blood circulation. So, more than 90% of inhaled formaldehyde is absorbed in the upper respiratory tract when formaldehyde is inhaled (IARC, 2006). Human exhaled air contains formaldehyde in a concentration range of 1 – 10 µg/m^3, with an average value of about 5 µg/m^3 (WHO, 2010). Despite its character and role as an endogenously-produced metabolic intermediate and a biosynthesis building block, there is reliable experimental and epidemiological data proving that inhalative exposure to formaldehyde can induce squamous cell carcinoma in the nasal cavity of rats and nasopharyngeal cancer in humans, respectively (cf. section 3.3.7.). Long-term exposures to 7.5 mg/m^3 (6 ppm) and above causes squamous cell carcinoma in the nasal cavity of rats with a non-linear biphasic concentration–response relationship having the break point at or above 2.5 mg/m^3 (2 ppm). In humans, no excess nasopharyngeal cancer has been observed at mean exposure levels of ≤1.25 mg/m^3 (~1 ppm) or with peak exposures <5 mg/m^3 (WHO, 2010). Considering all experimental animal data and epidemiological human data, IARC has evaluated and classified formaldehyde as a “known human carcinogen” (Group 1; IARC, 2006). Moreover, IARC causally associated inhalation exposure to formaldehyde not only with nasopharyngeal cancer but with the occurrence of human leukaemia as well (IARC, 2012). In addition, ECHA’s Committee for Risk Assessment concluded in 2012 that a harmonised classification as Carc Cat 1B H350 (“may cause cancer”) is appropriate (RAC, 2012).

Besides skin, formaldehyde is also an irritant of the eyes and of the respiratory tract. In 2010, a WHO working group reassessed and confirmed an earlier derived Indoor Air Guideline Level based on the following observation: “Increases in eye blink frequency and conjunctival redness appear at a concentration of 600 µg/m$^{3"}$, which was then considered the NOAEL. There is no indication of accumulation of effects over time with prolonged exposure. The perception of odour may result in some individuals reporting subjective sensory irritation, and individuals may perceive formaldehyde at concentrations below 100 µg/m^3. However, this is not considered to be an adverse health effect. The NOAEL of 600 µg/m^3 for the eye blink response is adjusted using an assessment factor of 5, derived from the standard deviation of nasal pungency (sensory irritation) thresholds, leading to a value of 120 µg/m^3, which has been rounded down to 100 µg/m^3 (0.08 ppm). Neither increased sensitivity nor sensitisation is considered plausible at such indoor concentrations in adults and children. This value is thus considered valid for short-term (30-min) duration, and this threshold should not be exceeded at any 30-min interval during a day. Thus, a short-term (30-min) guideline value of 100 µg/m^3 (0.08 ppm) is recommended to prevent sensory irritation in the general population. Thus, use of this short-term guideline value of 100 µg/m^3 will also prevent long-term health effects, including nasal and lymphohaematopoietic

cancers in humans." (WHO, 2010).

In 2006, a similar outcome originated from an evaluation conducted at the German Federal Institute for Risk Assessment (Schulte *et al.*, 2006). The authors used sensory irritation on eyes, nose and throat as surrogate for epithelial irritation and cytotoxicity, the latter of which being expected to proceed into a cancer risk. Schulte *et al.* concluded: "Concerning the tumors in the upper respiratory tract, the steps in the induction of tumors are understood and include non-genotoxic mechanisms, which in the low concentration range are the most critical events. Hence, it seems well founded that a safe level can be derived despite the fact that genotoxicity also plays a role in tumor formation. Our analysis of the available human data suggests that a level of 0.1 ppm (123 µg/m^3) formaldehyde is safe for the general population."

3.4.2. Inhalative exposure by release of formaldehyde in nail hardeners

Nail hardeners are a group of specialised products not intended to be used on a daily basis. These products are sold in small bottles labelled with warnings and instructions, predominantly that skin contact should be avoided or that the skin should be protected with grease, oil or other nail shields. Given the different usage instructions, the number of applications may be at maximum 124 times/year (cf. section 3.2.).

Special investigations

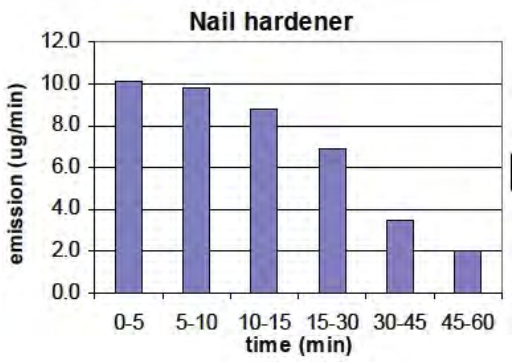
Consumers may inhale formaldehyde following the volatilisation of this compound from the use of nail hardeners, although studies evaluating this risk are rare. The following three studies provide at least some data on this issue. Among these, the TNO study was conducted according to GLP with a small number of human volunteers in a bathroom under real application conditions.

1st study: TNO, 2008 (not published)

Guideline:	ICH Guideline for Good Clinical Practice (1996/1997) with approved protocol by Medical Ethics Committee (2006). Open study with application of one commercially available nail hardener.
Species:	Human (female volunteers)
Group size:	6 females per group (non-smokers)
Test substance:	Commercial nail hardener (viscous fluid) containing formaldehyde as active ingredient
Batch:	602 (nominal formaldehyde concentration: 2.2%)
Route:	Single topical application to the nail plate (10 fingernails)
Dose level:	1 mg/cm^2 (based on the dermal area dose for leave-on products)
Exposure period:	60 min
Product application:	Product application was done in a small bathroom (9.4 m^3) with a ventilation rate of 5 times/hour (about 47 m^3/h) at a temperature of about 23°C and relative humidity of 30 – 50%. The intended target amount was 0.25 g nail hardener/volunteer. After finishing the application, individual measurements of free formaldehyde released from the products were carried out by applying air sampling devices (DNPH-cartridges connected to pumps operating at 1.4 l/min) which were mounted in close vicinity of the individuals (breathing zone) before, during and after one hour of product application. Prior to application, bathroom and volunteer blank values were determined by static air sampling (SAS) and personal air sampling (PAS) for 30 min each. All results were corrected for these individual background levels of formaldehyde, measured prior to product application. The volunteers were physically examined for any adverse health effects during the study.

Sampling:	Study in model bathroom: bathroom blanks (for 30 min), volunteer blanks (for 30 min), and sampling intervals of 0–5, 0–10, 0–15, 0–30, 0–45, 0–60 minutes following application. Background blanks by SAS (static air sampling, bathroom) and individual blanks by PAS (personal air sampling, volunteer at breathing zone) were performed by using the same devices as for the actual measurments upon product application. Specific emission rates (SERm) of formaldehyde were measured in a test chamber (86 cm x 65 cm x 36 cm = 200 l; 1 m^3/h,, 5/h) in accordance with DIN EN 13419.
Analysis:	Determination of free formaldehyde content in the commercial nail hardener according to the validated TNO Standard Operating Procedure (SOP) COS/013 as the official EU method 82/434/EEC. Formaldehyde was trapped using commercially available DNPH-coated cartridges connected via air sampling lines and mounted in the vicinity of the breathing zone to pumps (operating at 0.9 l/min). The formaldehyde-DNPH adducts were eluted with acetonitrile and measured by HPLC and UV detection.
GLP:	yes

Results:
1) Formaldehyde concentration in the test formulation (nail hardener): 2.17% ± 0.08%.
2) Test chamber study: The specific emission rates (SERm, [µg/(g x h)] of formaldehyde from the nail hardener was determined at 15,725 ± 5,884 µg/(g x h). Calculation of SERm is described in DIN EN 13419. It is defined as normalised value irrespective of the amounts applied, the dimensions of the chamber, air exchange rates, etc. These results expressed as emission rates (µg/min) showed that the amount of formaldehyde released declined to the fifth of the original level within 45 – 60 minutes:



3) Bathroom study: The bathroom blank measurements (SAS), determined 30 min prior to subjects entering the bathroom (1.64 ± 0.89 µg/m^3), and the subjects blank measurements (PAS) observed 30 min at rest sitting on a chair in the bathroom (2.79 ± 0.84 µg/m^3), were determined to be generally lower than the values of the test volunteers. The maximal exposure to free formaldehyde released from the commercial nail hardener was 74.9 ± 15.4 µg/m^3, with an interindividual range of 10.3 – 97.1 µg/m^3. So, the highest individual maximal mean free formaldehyde concentration established was 97.1 µg/m^3. These maximal concentrations were reached in about 5 minutes after application and declined towards background levels within 45 – 60 minutes.

2nd study: Kelly *et al.* (1999) measured the emission rates of formaldehyde from materials and consumer products found in California homes, including nail hardeners. Items were tested unter typical indoor conditions in a test chamber (1.43 m^3). Product loading was about 15 – 16 mg/m^2. Among all products tested, nail hardener exhibited the second highest initial emission rates per cm^2 of product surface (R_o), that is c_{max} of 180 – 195 µg/m^3 under typical conditions (21°C, 50% RH, 1/h) and 255 – 295 µg/m^3 under “elevated” conditions (27°C, 50% RH, 1/h). Under these typical conditions initial and final

formaldehyde emission rates were determined at 178,000 – 253,000 and 124 – 471 µg/(m^2 x h), respectively. Thus, the final rates (measured after 24 h) were 0.2% or less of the initial rates.

3rd study: Based on the emission rates published by Kelly *et al.*, 1999, the German Federal Institute for Risk Assessment (BfR) used model calculations according to the CONSEXPO programme and assessed the contribution of nail hardeners containing 5% formaldehyde to the overall exposure against this compound in indoor air (BfR, 2005). Usage of 0.5 g product for 30 min in a 20 m^3 bathroom, applied on a nail area of 0.002 m^2 (20 cm^2), that is 25 mg/cm^2, were calculated to cause mean-event concentrations for non-users and users of about 1.7 and 6.8 µg/m^3, respectively. Based on these comparably low numbers and the assumption of immediate distribution of gaseous formaldehyde in the ambient air, BfR concluded that the short-time peak reached by nail hardener products can be considered as insignificant given the overall background exposure levels present in buildings (147 µg/m^3 formaldehyde constituting the upper value not being exceeded by more than 95% of all German households; cf. below).

Discussion of exposure of consumers applying nail hardeners

Inhalation of formaldehyde may occur following the volatilisation from the use of nail hardeners. A GLP study mandated by the cosmetics industry delivered data on the exposure of healthy human female volunteers to formaldehyde released from nail hardeners during application (cf. above; TNO, 2008). In this study, under daily life conditions, the maximum formaldehyde concentration in ambient air was reached within 5 min after usage (about 74.9 ± 15.4 µg/m^3 with maximal levels of 97.1 µg/m^3) and declined rapidly to low levels within 45 – 60 minutes. So, the 30-min average value was measured at ~45 µg/m^3, whereas the mean average value for the entire period of 0 – 60 min was in the range of 20 – 30 µg/m^3.
All of these values lie below both the “safe level” of 123 µg/m^3 (100 ppb) derived by Schulte *et al.* in 2006, and the air quality guideline value of 100 µg/m^3 (as a 30-min average value) recommended by the WHO in 2010. However, assuming regular background levels in indoor air, which—according to the European AIRMEX database—are in the range of 24.1 µg/m^3 (with 95th percentile values of 52.4 µg/m^3), the overall formaldeyde exposure for consumers applying nail hardeners in small bathrooms might approach the 30-min average level. At the same time a short-time exceedance of the level of 100 µg/m^3 formaldehyde might occur within the first few minutes directly after application of a nail hardener product that contains 2.2% formaldehyde. Applying an even more conservative assessment by using the “worst case” background levels of 85 µg/m^3, a value suggested via REACH registration dossiers (see section 3.7. below), initial concentrations of formaldehyde in close proximity of nail hardener application areas may even reach peak levels of up to 180 µg/m^3 right after application (0 – 5 min) and *average levels* for the initial 30-min period of 130 µg/m^3. According to the numbers provided, the average formaldehyde level for the entire period of 60 min after nail hardener application will be in the range of 100 µg/m^3 (background level included).

Although the regulatory basis for protecting consumers against adverse health effects originating from exposure to cosmetics differs from the corresponding regulation of such chemicals in occupational settings, occupational exposure limits (OELs) such as TWAs (time-weighted averages for an 8-hour work shift), STELs (short-term exposure limits for a 15-min period), and TVLs (treshold level values) or so-called “ceiling limits” (maximal exposure limit which is not to be exceeded even momentarily) might be useful to put the above-mentioned values into context:

Country/ Institution:	TWA:	STEL:	TLV:
Germany	0.3 ppm (370 µg/m^3)	0.6 ppm (740 µg/m^3)	1 ppm (1228 µg/m^3)
France	0.5 ppm (614 µg/m^3)	1 ppm (1228 µg/m^3)	
UK	2 ppm (2456 µg/m^3)	2 ppm (2456 µg/m^3)	
Netherlands	1 ppm (1228 µg/m^3)	1.5 ppm (1842 µg/m^3)	
USA	0.75 ppm (921 µg/m^3)	2 ppm (2456 µg/m^3)	
SCOEL	0.2 ppm (246 µg/m^3)	0.4 ppm (491 µg/m^3)	

Sources: http://www.worksafebc.com/regulation_and_policy/policy_decision/board_decisions/2009/july/assets/formaldehyde.pdf and recommendation from the European Commission's Scientific Committee on Occupational Exposure Limits (SCOEL) for formaldehyde (SCOEL/SUM/125, March 2008).

The duration of formaldehyde exposure for the application in nail hardeners is less than one hour (background levels restored within 45 – 60 min). The peak levels of formaldehyde being released will be reached immediately within the first minutes after treating the nails. Under some circumstances, these maximum levels might shortly approach the WHO air quality guideline concentration of 100 µg/m^3 (0.0815 ppm), but—if so—only for a few minutes and only to an extent well below of any of the OELs currently in force at the international level. Among them, the most restrictive OELs, as suggested by SCOEL, would tolerate work place concentrations of 250 µg/m^3 (0.2 ppm) for 8 h/day and 5 days/week and 500 µg/m^3 (0.4 ppm) for any 15-min short-term exposure period (see Table above). All of these values are expected to adequately protect workers from adverse health effects mediated by inhalative exposure to formaldehyde. On the other hand, it requires mentioning that – besides the well-established WHO guideline value of 100 µg/m^3 – certain countries such as France are currently discussing the suggestion of reducing the acceptable levels for long-term formaldehyde exposure to a value of no more than 30 µg/m^3 (JORF, 2011).

3.4.3. Aggregate inhalative exposure of the entire human population

The aggregate exposure of humans to formaldehyde has been exhaustively reviewed over a long period of time by several expert groups (e.g., IARC, 2012). Formaldehyde occurs naturally, including in some foods, and is formed endogenously in mammals, including humans as a consequence of oxidative metabolism (EFSA, 2014). In addition to these natural sources, common non-occupational sources of exposure to formaldehyde include combustion processes, e.g. through emissions from motor vehicles, power plants, incinerators, refineries, wood stoves, and kerosene heaters (Salthammer *et al.*, 2010). Formaldehyde may be released from particle boards and similar building materials, carpets, paints and varnishes, during the cooking of some foods, and during its use as a disinfectant. It is also present in tobacco smoke. An indirect source of exposure to formaldehyde is its formation via photochemical oxidation of hydrocarbons, such as methane, and other precursors emitted from combustion processes (NTP, 2005; IARC, 2012).
Formaldehyde has a short half-life in the environment. It is removed from the air by photochemical processes and by precipitation and biodegradation (NTP, 2005). Concentrations of formaldehyde in outdoor air are generally below 1 µg/m^3 in remote areas and below 20 µg/m^3 in urban settings. The levels of formaldehyde in the indoor air of houses are typically 20 – 60 µg/m^3; indoor combustion sources can significantly increase these levels. Cigarettes may contribute as much as 10 – 25% of the indoor exposure. Besides all of these environmental sources, formaldehyde is present in a variety of consumer products including cosmetics (NTP, 2005).

Nielsen *et al.* (2013) summarised the overall environmental exposure to formaldehyde as follows: Major indoor air sources are building materials (e.g. wooden products as furniture, particleboard, plywood and medium-density fibreboard), consumer products and combustion processes (WHO, 2010). Indoor air is the dominating contributor to formaldehyde exposure through inhalation. Therefore, WHO developed an indoor air guideline value in 2010. The critical effects were considered the portal-of-entry effects, sensory irritation of the eyes and the upper airways, resulting in a guideline value of 100 µg/m^3 that should not be exceeded for any 30-min period of the day. The guideline intends to prevent sensory irritation after acute and chronic exposure and cancer. In Europe, Canada and the US, the general mean formaldehyde levels in homes or dwellings were generally within 20 – 40 µg/m^3, the 95th percentiles were roughly two times higher. Higher mean concentrations have been reported in China (~240 µg/m^3). In Europe and the US, mean ambient outdoor concentrations were typically in the range 1 – 4 µg/m^3, with higher levels in polluted cities (WHO, 2010). According to Salthammer *et al.* (2010), the general levels in homes and dwellings in the Northwestern hemisphere were found in the range of 10 – 80 µg/m^3), with the 95th percentiles mostly ≤100 µg/m^3.

Assessing the years between 1985 and 2004, a study from the German BfR suggested that a level of 147 µg/m^3 formaldehyde would be the upper value not exceeded by more than 95% of all German households (BfR, 2005). Based on emission studies by Kelly *et al.*(1999), the most important sources of formaldehyde release discussed were wooden flake boards (coated with formaldehyde resins), paintings, crease-resistant textiles, adhesives used in paper products and certain cosmetics. Newer data indicate that the overall indoor exposure level has been decreasing since these studies were conducted.

Aggregate exposure to formaldehyde from cosmetics including background levels has been estimated by Cosmetics Europe using a “Probabilistic Exposure Model” with data input from various sources, including the COLIPA/TNO consumer inhalation exposure study (cf. above; TNO, 2008, Lefebvre *et al.*, 2012). The model estimated the exposure to formaldehyde from cosmetic products.
Details of this study: For probabilistic exposure modelling, information about the frequency and mode of cosmetic product consumption is necessary. These data were obtained from a database of the habits and practices of cosmetics consumers to simulate a realistic consumption pattern across the European population (“Creme Global database”). Herein, the use of cosmetics is described at a time interval of one hour or more. Although nail hardener products are not included in the Creme Global database, they were integrated in the calculation (cf. below). The model applied considers every one-hour-long usage event reported in the database as an independent consumption event.
The exposure model also required data on the quantity of formaldehyde contained in and released by the cosmetic products into ambient air. These data were retrieved from two TNO bathroom experiments and from Lefebvre *et al.* (2012). The first TNO bathroom experiment assessed the concentration levels of formaldehyde released in the air by different cosmetic products, while the second experiment assessed nail hardener products only (cf. above; TNO, 2008). The exposure model also considered the background formaldehyde exposure levels originating from other sources, e.g. formaldehyde released by each individual subject, their clothes, furniture and other external sources (“background formaldehyde”). Formaldehyde in medications or food, however, were excluded since these sources do not significantly contribute to the air-borne exposure. Different levels of formaldehyde present in European homes were simulated according to the concentration levels reported in the European Indoor Air Monitoring and Exposure (AIRMEX) database. Formaldehyde present in the room of use and released by cosmetic products including nail hardeners and products containing formaldehyde releasers was then aggregated to estimate the possible concentrations of formaldehyde in the air. Sources used for the probabilistic exposure model (summary):
1. Creme Global database: Consumer habits and practices for cosmetics
2. AIRMEX database: Concentration level of background formaldehyde in the air

3. TNO studies: Cosmetic product amounts used, quantities of formaldehyde in the product and emitted into ambient air
4. Code-Check database: Probability that individual cosmetic products contain formaldehyde

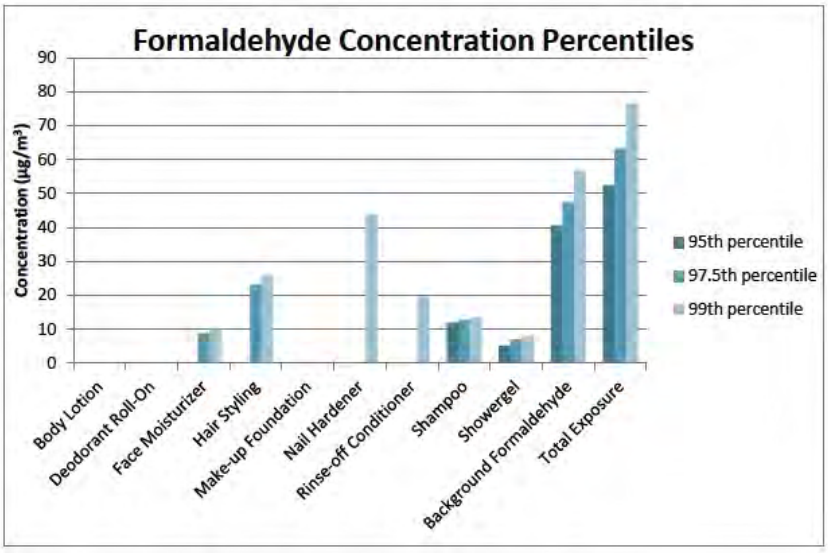
Formaldehyde presence probabilities for each category of cosmetic product were estimated on the basis of the online Swiss Code-Check database (www.codecheck.info). The probabilistic exposure model randomly selected the products containing formaldehyde from a category according to the probability for that product category. The following further assumptions with regard to cosmetics were made:

1. The concentration limit of total free formaldehyde in cosmetic products, including that contained in the formaldehyde releasers, is fixed to 0.2% (Annex V of the Regulation (EC) No 1223/2009) with the exception of oral care products (0.1%, Annex III of the Regulation (EC) No 1223/2009) and of nail hardeners set equal to 2.2%.
2. It was assumed that 30% of all women in the above-mentioned database (randomly selected) use nail hardeners due to the reasoning that this percentage of women over 50 years is affected by nail brittleness.*
3. The predominant usage frequency is around 65 times/yr for all consumers, i.e., about 1.25 times per week.*
4. Nail hardeners are supposedly used in parallel with other cosmetics in the bathroom.*
5. Formaldehyde releasers were included in the exposure model and considered as “free” formaldehyde.
6. Only adults were considered.

The probabilistic exposure model simulated in total 100,000 subjects representative for the European population and estimated the exposure levels for each consumption event that lasted one hour (in total more than 800,000 consumption events). For nail hardeners (used once a week by only 30% of all female subjects listed) this means that only about 15,500 consumption events are included (about 2% of total). In consequence, the 95th and 97.5th percentile of nail hardener exposure in the total population was equal to zero. Therefore, the 99th percentile was necessary to show the contribution of the nail hardener products (see Figure below).
The model calculated the following values for total formaldehyde exposure, background formaldehyde exposure and aggregate levels of formaldehyde released by cosmetic products (mean formaldehyde concentrations per consumption event for every consumer in the simulation of 100,000 subjects):

Formaldehyde exposure	**Cosmetic products**	**Background**	**Total aggregate exposure**
	(μg/m^3)		
Mean	4.2	19.9	24.1
95th percentile	23.9	40.6	52.4
97.5th percentile	33.0	47.4	63.3
99th percentile	50.8	56.8	76.5

Aggregate levels of formaldehyde were disaggregated to different cosmetic product categories. Comparison of the upper percentiles of formaldehyde concentration distribution released by the individual cosmetic products in relation to background exposure revealed that nail hardener products were placed within the 99th percentile:



Altogether, the model calculated a value of 4.2 µg/m^3 for the mean aggregate formaldehyde exposure from all cosmetic products including those from nail hardeners. According to the assumptions made, nail hardener products are used by only 30% of women and only once per week. This leads to events with nail hardener application accounting for only about 2% of the total exposure. The concentrations of formaldehyde released in the air by these products rapidly decrease towards the background levels. Nonetheless, products with high formaldehyde concentration levels like nail hardeners release more formaldehyde; in this case their contribution in the average concentration of released formaldehyde and in the upper percentiles will become significant even if they are rarely consumed. Therefore the 99th percentile of nail hardeners becomes much higher than the percentiles of the other cosmetic products.

Conclusions drawn from this particular modelling study: For the entire population, a mean aggregate formaldehyde exposure in the air was estimated at 24.1 µg/m^3, with a 95th percentile value of 52.4 µg/m^3 for varying concentrations over time including background levels. For 99% of all cosmetic consumption events, this concentration is lower than 76.5 µg/m^3. The background exposure to formaldehyde (by other sources than cosmetics) accounted to a mean aggregate level of 19.9 µg/m^3 with a 95th percentile value of 40.6 µg/m^3. Conversely, the model calculated a value of 4.2 µg/m^3 for the mean aggregate formaldehyde exposure from all cosmetic products including nail hardeners. As nail hardener products were assumed to be used by only 30% of women only once per week, the simulated events with nail hardener applications account for only about 2% of the total exposure and consequently, the 95th and the 97.5th percentile of nail hardener exposure in the total population are very low. Only after applying the 99th percentile could the contribution of the nail hardener products to the overall exposure be determined under these conditions.

3.4.4. Estimates on the overall inhalation exposure of consumers (according to REACH registration dossiers)

According to Regulation (EC) No 1223/2009, an overall exposure has to be considered in for the safety assessment of CMR 1A or 1B substances: This overall exposure should consider all sources. Accordingly, an estimate on the overall exposure of consumers to formaldehyde has been retrieved from REACH registration dossiers submitted to the European Chemicals Agency (ECHA). The information obtained from there can be summarised as follows:

- Average and worst case indoor air inhalation exposure have been assessed by use of measured data described in the literature. Measurements were all taken indoors, with a varying description of determinants. Based on a data set of >2500 measurements, the central tendency of formaldehyde indoor air concentration in Europe turned out to be

around 25 µg/m^3. Conversely, the reasonable worst case level as determined from all data was estimated at 85 µg /m^3**.**

- In newly built houses or due to renovations or redecoration the formaldehyde indoor air concentration may be increased. Literature indicates that even in these situations the indoor concentrations tend to be below 100 µg/m^3 (that is, current REACH DNEL).
- Measurements in new homes do (probably temporarily) show slightly elevated levels of formaldehyde. Furthermore, the emission rates of different product types have been compared and show a large range, even within a product category. The testing method has a relatively large influence on the test result. The results indicate that the main sources of formaldehyde emission are the uncoated materials and plywood, whereas products like paints, mineral wools and foams have lower emissions. In general emissions decrease over time.
- Under laboratory conditions (chamber testing), paints and other products might result in peak concentrations of >100 µg/m^3.
- A reasonable worst-case exposure scenario of a wardrobe in a European reference room with both ceiling and floor made up of wood-based products resulted in a maximum formaldehyde concentration of 93 µg/m^3.

Taking all data and estimates into account, it can be concluded that the general concentration of formaldehyde in homes, as well as a reasonable worst case estimate of exposure in new homes, are below the reference value of of 100 µg/m^3.

However, due to the limited information on the real exposure levels of consumers to formaldehyde, provided by industrial dossiers at ECHA (and as extracted from the literature), this compound has been put on the Community Rolling Action Plan (CoRAP) based on the following reasoning: “It is not possible to come to definitive conclusions on the risks for consumers and workers because essential information is missing. The following information is needed to make a more in-depth risk assessment:

- Workers' exposure concentrations for all processes covering the whole life cycle.
- Measured indoor air exposure data for consumers in different European countries, taking into account worst-case scenarios: newly built house with new kitchen, furniture, curtains and carpet.
- Data on how long high concentrations of formaldehyde in indoor air from new material (new kitchen, furniture, curtains and carpet) persist.

(cited from: http://echa.europa.eu/documents/10162/a76a77ad-dbb9-46e1-8121-8f2931f71fa3)

3.5. Safety evaluation (including calculation of the MoS)

The safety evaluation is based on a comparison of exposures to formaldehyde from nail hardeners with generally accepted indoor air limit values derived by WHO in 2010 (cf. section 3.4.1.). Neither increased sensitivity nor sensitisation is considered likely at such indoor concentrations in humans. This threshold value was thus considered valid for short-term duration and consequently should not be exceeded at any 30-min interval during a day. According to WHO, the use of this air quality guideline value of 100 µg/m^3 will also prevent long-term health effects, including nasal and lymphohaematopoietic cancers in humans.

3.6. Discussion

Although the contribution of cosmetics to the aggregate exposure of consumers against formaldehyde seems rather low (cf. section 3.6.), application of nail hardeners with a maximum content of 2.2% free formaldehyde are likely to result in short-time inhalative exposure of consumers of >100 µg/m^3 (cf. section 3.5.). By including “worst case” formaldehyde background levels as high as 85 µg/m^3, peak values may even reach 180

µg/m^3 right after regular application of products that contain 2.2% formaldehyde (note: according to the European AIRMEX database, regular formaldehyde background levels are in the range of 24 µg/m^3).
Based on the most sensitive effect of formaldehyde in humans (eye irritation), the level of 100 µg/m^3 (0.0815 ppm) has been suggested by WHO in 2010 as a safe indoor air guideline value not to be exceeded for any 30-min period of the day. Under these conditions, this threshold is assumed to effectively protect humans against sensory irritation and cancer mediated by inhalative exposure to formaldehyde (cf. section 3.4.). Some years earlier, considering the same scenarios (sensory irritation on eyes, nose and throat as surrogate for epithelial irritation and cytotoxicity), a level of 123 µg/m^3 (0.1 ppm) was suggested as being safe for the general population (Schulte *et al.*, 2006). Although the "worst case" estimates may shortly exceed the WHO guideline value by almost a factor of 2, formaldehyde levels in ambient air were shown to decrease rapidly to background levels after nail hardener application. So, the *average* 30-min and 60-min period levels after 2.2% formaldehyde application lie in the range of about 45 and 25 µg/m^3 (without background), respectively (cf. section 3.4.2.). It should be noted, however, that these values measured in an appropriate setting (bathroom study) are based on the application of nail hardener to the 10 fingernails only. In this particular study there was no mention of an application to the toenails at the same time, which might be considered possible. However, currently no experimental data are available on this issue, and corresponding and reliable information on the actual consumer behavior in terms of nail hardener usage is missing.
The duration of formaldehyde exposure after application of nail hardeners is less than one hour (background levels restored within 45 – 60 min). Within this time window, peak levels in close proximity to the application area will be reached immediately after treating the nails. Although the maximum levels possible might shortly exceed the WHO air quality guideline level of 100 µg/m^3 to some degree, this will last only for a few minutes and only to an extent well below any of the internationally accepted exposure limits that are expected to effectively protect humans from adverse health effects mediated by inhalative exposure to formaldehyde at work places. The most restrictive limit value currently in force is 500 µg/m^3 for any 15-min short-term exposure period during a day (see 3.4.2.).

Formaldehyde is a known skin sensitiser. The skin sensitising properties of formaldehyde have been addressed in numerous studies. In animal studies (LLNA in mice), formaldehyde revealed as a strong dermal sensitizer (cf. section 3.3.3.). The lowest EC_3 value established was at 0.29% formaldehyde. By contrast, in humans a threshold concentration for sensitisation induction has been estimated to be less than 5%, with positive responses already starting at 1% formaldehyde (4.5% of the patients; cf. section 3.3.10.3.). In selected populations (i.e., clinical patients), approximately 2% showed a positive reaction to 1% formaldehyde in water. Based on these patch tests, a threshold concentration for the elicitation in already sensitised individuals has been suggested at 30 ppm (0.003%) in aqueous solution and 60 ppm (0.006%) for products containing formaldehyde (OECD, 2002). Others suggested positive reactions to formaldehyde are rather rare at concentrations below 0.025 – 0.05% (ATSDR, 1999).
Given these numbers, the application of nail hardeners that contain maximum concentrations of 2.2% formaldehyde is required to be performed very carefully and with caution. Although cosmetic products that contain formaldehyde in a concentration above 0.05% should be labelled with the warning to "protect cuticules with grease or oil" (Regulation (EC) No 1223/2009), some formaldehyde may reach the skin surrounding the nail if the product is not carefully applied on the nail plate or not handled according to the instructions. In addition, there is some chance that formaldehyde might reach living tissue if nail hardeners would be applied on severely damaged or broken nail plates. Since there is occasional evidence that formaldehyde in nail hardeners might lead to the induction or elicitation of dermatitis around the finger nails but also at distant sites (Andersen *et al.*, 2008), proper handling and usage of these products becomes mandatory. On the other hand, to achieve the effects desired, nail hardeners do not seem to necessarily require formaldehyde concentrations as high as 2.2%, given the products on the market that contain much less of this compound (<1%, Andersen *et al.*, 2008).

3.7. Conclusion

In view of above, and taken into account the data provided in the application, and from other sources, SCCS is requested:

1) to assess if the use of formaldehyde in nail hardeners at the maximum level of 2.2% (as free formaldehyde) can be considered safe or not

Nail hardeners with a maximum content of about 2.2% free formaldehyde can be used safely to harden or strengthen nails. Although “peak values” of formaldehyde reached in ambient air surrounding the application area may approach the WHO indoor guideline value of 100 µg/m^3 formaldehyde (30 minutes exposure), thereby exceeding this concentration level only for a short period immediately after application, formaldehyde levels will rapidly decrease to background levels again within a few minutes.
The SCCS has concerns about the sensitisation potential of nail hardeners containing formaldehyde (see question 2).

2) to indicate if there are any further scientific concerns with regard to the use of formaldehyde in nail hardeners.

In order to reduce inhalation exposure to formaldehyde, the room should be ventilated when applying nail hardeners.

The risk of local effects in the skin, such as sensitisation, can be minimised if the products are used properly and according to the present EU Cosmetics Regulation (including the phrase ‘protect cuticules with grease or oil’). In light of the very low concentration of formaldehyde that has been suggested to be capable of elicitating allergy in already sensitized individuals (>0.006%), careful usage and handling of nail hardeners is recommended. Severely damaged nails should not be exposed to nail hardeners containing formaldehyde.

Risk for professionals who offer application of nail hardeners as part of their service and therefore may be more frequently exposed to formaldehyde has not been assessed in this Opinion.

ANNEX

4. TOXICOLOGICAL EVALUATION

4.1 Acute toxicity

4.1.1 Acute oral toxicity

1st study: Tsuchiya *et al.*, 1975

Guideline:	comparable to OECD TG 401
Species/strain:	Rat (Wistar)
Group size:	6 – 16 males per group
Test substance:	Formalin (37% formaldehyde in aqueous solution, stabilised with 10% methanol) diluted to 2% or 4% solutions
Batch:	no data
Purity:	no data
Vehicle:	n/a
Dose levels:	2% solution: 230, 300, 400, 520, 675 mg/kg bw (max. 33 ml/kg) 4% solution: 400, 520, 675, 875, 1140 mg/kg bw
Administration:	oral (gavage)
GLP:	no
Study period:	1 week (overall observation period 3 weeks)

Results:
Rats died mostly within 24 hours after application. LD_{50} (determination via Litchfield method; Litchfield and Wilcoxon, 1949): 460 mg/kg bw (2% solution) or 832 mg/kg bw (4% solution)

2nd study: Smyth *et al.*, 1941

Guideline:	comparable to OECD TG 401
Species/strain:	Rat (Wistar) and Guinea pigs
Group size:	10 males/group (rats), 10 males & females/group (guinea pigs)
Test substance:	Formalin, 2% (no further data)
Batch:	no data
Purity:	no data
Vehicle:	n/a
Dose levels:	max. concentration fed: 2%, no further data
Administration:	oral (gavage)
GLP:	no
Study period:	2 weeks

Results:
Rats and guinea pigs died mostly within 24 hours after application. LD_{50} (determination via Method of Probit described by Bliss): 800 mg/kg bw (rats) and 260 mg/kg bw (guinea pigs), respectively.

4.1.2 Acute dermal toxicity

According to a reference cited by the WHO (1989, see table below), the dermal LD_{50} in rabbits was reported to be 270 mg/kg bw. However, according to the "NIOSH Skin Notation (SK) Profile on formaldehyde / formalin" from April 2011, no animal dermal LD_{50} value has been identified so far (see: http://www.cdc.gov/niosh/docs/2011-145/pdfs/2011-145.pdf).

TABLE 3. Acute LD_{50} Values for Formaldehyde in Various Species.

Species	*Route*	*LD_{50} (mg/kg)*	*Ref.*
rat	oral	800	72
	s.c.	420	73
	i.v.	87	74
mouse	s.c.	300	73
rabbit	dermal	270	75
guinea pig	oral	260	72

Source: "Final Report on the Safety Assessment of Formaldehyde", Int. J. Toxicol. 3: 157-184 (1984)

4.1.3 Acute inhalation toxicity

1st study: Skog, 1950

Guideline:	comparable to OECD TG 403
Species/strain:	Rat (no further data)
Group size:	8 per group
Test substance:	Formalin (35.5% formaldehyde in aqueous solution)
Batch:	no data
Purity:	no data
Vehicle:	n/a
Concentration levels:	600 – 1700 mg/m^3 (9 dose groups)
Administration:	Inhalation (whole body)
Exposure:	30 min
GLP:	no
Study period:	3 weeks (overall observation period 3 weeks)

Results:
The rats became listless and showed lacrimation, secretion from the nose, wheezing and rattling respiration, gasping; respiration difficulties lasted for several days; last death as late as the 15th day after exposure. Pathology revealed haemorrhages and oedema in lungs, hyperaemia, perivascular oedema, necrosis in the liver and perivascular oedema in the kidney. Thus, local (respiratory system) as well as systemic effects (liver, kidney) and delayed mortality were observed. The LC_{50} (30 min) value was 1,000 mg/m^3.

2nd study: According to a secondary reference (OECD, 2002), Nagorny *et al.* (1979) exposed rats (21 groups) at nominal concentrations between 230 and >900 mg/m^3 for 4 hours. The rats showed symptoms consisting of restlessness, excitation, laboured breathing, gasping and lateral position. A fraction of animals died at 390 — 940 mg/m^3, and nearly all animals

died at concentrations >900 mg/m³. The LC_{50} (4 h) value was calculated at 578 mg/m³ (480 ppm) (see table below).

Summary on acute toxicity:

Table 3.1-2 Acute toxicity of formaldehyde

Species	Route		Reference
Rat	Oral	LD_{50} 600 – 700 mg/kg body weight	Tsuchiya K. *et al.* ,1975
Rat	Oral	LD_{50} 800 mg/kg body weight	Smyth *et al.*, 1941
Rabbit	Dermal	LD_{50} 270 mg/kg body weight	WHO IPCS 1989 [1]
Rat	4 h inhalation	LC_{50} 578 mg/m^3 (480 ppm)	Nagorny *et al.*, 1979
Rat	30 min inhalation	LC_{50} 984 mg/m^3 (816 ppm)	Skog, 1950

[1]No further details were available. Secondary literature; reliability was not assignable

Source: "Initial Assessment Report Formaldehyde" (OECD, 2002)

In rats, the acute oral LD_{50} was 600 – 800 mg/kg bw and the LC_{50} (4 h) in inhalation studies was 578 mg/m³ (480 ppm). However, inhalation exposure studies in mice provided evidence that they seem more sensitive than rats. A 50% reduction in the respiratory rate resulted from exposure to between 3 and 5 ppm formaldehyde in mice whereas 10 – 30 ppm was necessary in rats (DFG, 2000).

4.1.4 Irritation and corrosivity

4.1.4.1 Skin irritation and corrosion

Study: Sekizawa *et al.*, 1994
Guideline: no
Species/strain: Rat (Wistar), Mouse (ddy), Guinea pig
Group size: 3 males and 3 females/group (rats and mice), 3 males/group (guinea pigs)
Test substance: Formaldehyde
Batch: no data
Purity: no data
Vehicle: water
Dose level: a. Rats: 3%, 7 – 9%, 15 – 18%, 37%
b. Mice: 7 – 9%, 15 – 18%, 37%
c. Guinea pigs: 7 – 9%, 15 – 18%
Dose volume: 1 ml/kg bw
Route: Dermal, dorsal area of 3 cm x 4 cm (rats, guinea pigs) or 1 cm x 2 cm (mice) on the trunk (shaved intact skin, unoccluded).
Observation: 1 week
GLP: no
Study period: 1 week

Results and conclusions:
Rats: A concentration-dependent increase in skin erosions including rubor and oedema was observed at 7 – 9%, 15 – 18% and 37%. Thus, the undiluted 37% formaldehyde solution

was considered corrosive. By contrast, the 3% solution induced no skin irritation in male and female rats under study conditions.
Mice: A concentration-dependent increase in skin erosions was observed at 15 – 18% and 37%. Thus, the undiluted 37% formaldehyde solution was considered corrosive. By contrast, the 7 – 9% solution resulted in equivocal results (the experiment with male mice was repeated after showing effects in the first experiment, while females remained unaffected at 7 – 9% formaldehyde.
Guinea pigs: No effect on the skin of the treated males was noted at 7 – 9%, but skin erosions were observed at 15 – 18%.

Additional study by Weil *et al.*, 1971: Inter-laboratory comparison on the skin irritant effects of 2% formaldehyde revealed no such effects in rabbits at this concentration in 13 out of 14 laboratories.

4.1.4.2 Mucous membrane irritation / Eye irritation

1st study: Carpenter and Smyth, 1946

Guideline:	no
Species/strain:	Rabbit (no data)
Group size:	5 animals
Test substance:	Formaldehyde
Batch:	no data
Purity:	no data
Vehicle:	water
Dose level:	15% solution
Dose volume:	0.005 ml
Route:	instillation in the conjunctival sac of the eye
Observation:	18-24 hours after instillation
GLP:	no
Study period:	1 day

Results and conclusions:
Instillation of 0.005 ml of 15% aqueous formaldehyde solution resulted in damage of the eye. The findings were scored (according to the Draize method) and graded as 8, that is, indicative of severe eye irritation. According to the current EU classification scheme, formaldehyde has thus to be considered as corrosive to the eyes of rabbits.

2nd study: Sekizawa *et al.*, 1994

Guideline:	no
Species/strain:	Rat (Wistar) and Mouse (ddy)
Group size:	3 males/group (either rats or mice)
Test substance:	Formaldehyde
Batch:	no data
Purity:	no data
Vehicle:	water
Dose levels:	3%, 7 – 9% solution
Dose volume:	0.01 ml
Route:	instillation in the conjunctival sac of the eye
Observation:	1 week
GLP:	no
Study period:	1 week

Results and conclusions:

Rats: At 3%, opaque cornea occurred in one test but in the repetition it was absent. Other effects like erythema were not part of the scoring system. At 7 – 9% solution, an opaque cornea was observed. Thus, formaldehyde has to be considered as corrosive to the eye of rats. Mice: At 3% no alteration was observed. At 7 – 9% solution, an opaque cornea was observed. Thus, formaldehyde has to be considered as corrosive to the eyes of mice.

Additional study by Weil *et al.*, 1971: Inter-laboratory comparison on the eye irritant effects of 2% formaldehyde revealed no irritant effects in rabbits at this concentration in 18 out of 20 laboratories.

Summary on skin and eye irritation:

In rodents, formaldehyde induces skin erosions starting at concentrations of 7%. The data available suggest eye corrosiveness beginning at 3% aqueous formaldehyde (no study according to current guidelines).

4.1.5 Skin sensitisation

4.1.5.1 Guinea pig maximisation test (GPMT)

1st study: Kimber *et al.*, 1991

Guideline:	OECD TG 406
Species/strain:	Guinea pig (Dunkin Hartley)
Group size:	10 females, 4 females in the control group
Test substance:	37% aqueous formaldehyde (formalin)
Batch:	no data
Purity:	no data
Vehicle:	physiological saline
Induction:	Intradermal and topical
Challenge:	Topical (occlusive)
Induction protocol:	6 intradermal injections of 0.25% formalin (slightly irritant) with and without Freund's complete adjuvant (FCA) followed by dermal application of 10% formalin (mildly irritant) for 48 h (occlusive) at day 6-8.
Challenge protocol:	Once, 12 – 14 days after induction with the maximum non-irritant concentration of 2% formaldehyde with patch removal after 24 h.
GLP:	no
Study period:	2 – 3 weeks

Results and conclusions:
At challenge with 2% formalin, 9/9 positive skin reactions (mean erythema score: 1.7). In the control animals without induction application, no skin reaction was observed. Thus, intradermal injection of 0.25% formalin, followed by dermal application of 10% formalin, induced skin sensitisation. A second study (Hilton *et al.*, 1996), confirmed these observations.

2nd study: OECD, 2002

Guideline:	OECD TG 406
Species/strain:	Guinea pig (Pirbright white)
Group size:	Females (no further data)
Test substance:	37% aqueous formaldehyde (formalin)
Batch:	no data

Purity:	no data
Vehicle:	physiological saline
Induction:	Intradermal and topical
Challenge:	Topical (occlusive)
Induction protocol:	2 intradermal injections of 0.1 ml of 5% formalin with and without Freund's complete adjuvant (FCA) followed by dermal application of 5% formalin for 48 h (occlusive) at day 9 – 11.
Challenge protocol:	Twice, at days 22 and 36 after induction with 2% and 4% formalin with patch removal after 24 h.
GLP:	no
Study period:	5-6 weeks

Results and conclusions:
Intradermal injection of 5% formalin, followed by dermal application of 5% formalin, induced skin sensitisation. At the challenge concentration of 4%, all animals revealed with skin sensitisation, while at 2% only 80% and 25% of the investigated animals showed skin reactions at the 1st and 2nd challenge, respectively.

4.1.5.2 Buehler test

Study: Marzulli and Maguire, 1982

Guideline:	OECD TG 406
Species/strain:	Guinea pig (Dunkin Hartley)
Group size:	10 females in both substance and control group (2 replications)
Test substance:	37% aqueous formaldehyde (formalin)
Batch:	no data
Purity:	no data
Vehicle:	physiological saline
Induction:	Topical (occlusive)
Challenge:	Topical (occlusive)
Induction protocol:	On the left flank of animals, 0.5 ml of 5% formalin (covered with Blenderm® and fixed with Elastoplast® and adhesive tape) were applied for 6 h. This application was repeated after 7 and 14 days.
Challenge protocol:	Once, on day 28 after induction, the same procedure as used for induction was performed at the contralateral (right) flank for 24 h.
GLP:	no
Study period:	2-3 weeks

Results and conclusions:
At challenge with 5% formalin, none of the animals in either the control group or the induction group revealed positive skin reactions (0/30). Under the condition of this study dermal application of 5% formaldehyde caused no induction of dermal sensitisation in guinea pigs.

4.1.5.3 Local lymph node assay (LLNA)

1st study: Kimber *et al.*, 1991

Guideline:	OECD TG 429 (inter-laboratory validation)
Species/strain:	Mouse (CBA/Ca)
Group size:	4 animals/group
Test substance:	37% aqueous formaldehyde (formalin)
Batch:	no data
Purity:	no data
Vehicle:	acetone: olive oil (4:1)

Protocol:	0.025 ml was topically applied on the dorsum of both ears daily for 3 consecutive days. At day 4, [^{3}H]-labelled thymidine was i.v. injected and mice were sacrified 5 h later. Proliferation of lymph node cells was measured via ß-scintillation counting (mean dpm/node x 10^{-2}, incorporation of labelled thymidine in pooled lymph node cells).
Concentrations:	5, 10, 25% formalin, vehicle control
GLP:	no
Study period:	4-5 days

Results:
Topical treatment with formalin led to a significant increase in [^{3}H]-labelled thymidine incorporation. The results obtained clearly demonstrate the skin-sensitising potential of formalin down to the lowest concentration applied in this study (5%): SI ≥3 (3.7-9.0) at 5% formaldehyde.

2nd study: Basketter *et al.*, 2001

Guideline:	OECD TG 429
Species/strain:	Mouse (CBA/Ca)
Group size:	4 females/group
Test substance:	37% aqueous formaldehyde (formalin)
Batch:	no data
Purity:	no data
Vehicle:	acetone: olive oil (4:1)
Protocol:	0.025 ml was topically applied on the dorsum of both ears daily for 3 consecutive days. At day 5, [^{3}H]-labelled thymidine was i.v. injected and mice were sacrified 5 h later. Proliferation of lymph node cells was measured via ß-scintillation counting (mean dpm/node x 10^{-2}, incorporation of labelled thymidine in pooled lymph node cells).
Concentrations:	0.1, 0.5, 1, 5, 10% formalin, vehicle control
GLP:	no
Study period:	5-6 days

Results: Topical treatment led to a significant increase in [^{3}H]-labelled thymidine incorporation at ≥0.5% formalin. The EC_3 value (3-fold stimulation of proliferation) was 0.93% formalin (corresponding to 0.35% formaldehyde).

These results were essentially confirmed in a more recent study conducted by Ulker *et al.* (2013). Here, *ex vivo* BrdU labelling and cytokine production were used as measurable endpoints upon formaldehyde application at 0.10, 0.25, 0.50 or 1.00% (3 consecutive days). As a result, an EC_3 value of 0.29% formaldehyde was determined based on BrdU labelling and substantiated through accompanying alterations of cytokine levels.

4.1.5.4 Supplementary Information: IgE production as surrogate for respiratory tract sensitisation (via dermal route)

Study: Hilton *et al.*, 1996

Guideline:	no
Species/strain:	Mouse (Balb/c)
Group size:	6 animals/group
Test substance:	37% aqueous formaldehyde (formalin)
Batch:	no data
Purity:	no data

Vehicle:	Dimethyl formamide (DMF, for formalin), acetone: olive oil (4:1, AOO) for DNCB, TMA (positive controls)
Positive controls:	2,4-Dinitro-1-chlorobenzene (DNCB) as skin sensitiser, trimellitic anhydride (TMA, 25%) as respiratory allergen
Protocol:	0.050 ml of test solution was applied on both shaved flanks; 7 days later 0.025 ml of half-concentrated test solution was applied to the dorsum of both ears. 14 days after exposure, serum was prepared after cardiac puncture and serum IgE measured via ELISA.
Concentrations:	vehicle controls DMF, AOO, 1% DNCB, 25% TMA, 10, 25 and 50% formalin
GLP:	no
Study period:	3 weeks

Results:
The topical treatment with formalin as well as with DNCB as known contact allergen led to no increase in the concentration of serum IgE. By contrast, TMA, a substance with respiratory allergic activity, stimulates a significant increase in serum IgE concentrations.
Summary of the results:

Serum IgE concentration in mice after treatment with formaldehyde in comparison with DNCP (skin sensitizer) and TMA (respiratory allergen), mean µg/mL±SD

DMF vehicle	10% Formalin	25% Formalin	50% Formalin	AOO vehicle	DNCB	TMA
0.334±0.086	0.407±0.040	0.471±0.066	0.330±0.040	0.326±0.038	0.254±0.050	2.016±0.176*

*: p<0.001

Conclusions:
Based on the results reported, formaldehyde is unable to prompt a systemic IgE response and thus is unlikely to cause sensitisation of the respiratory tract via the dermal exposure route. This result was indirectly confirmed by another endpoint addressed in the same study (i.e., pattern of cytokine secretion in cells isolated from draining auricular lymph nodes). Topical application of formalin in mice led to the production of IFNγ (via T_H1), but did not increase the IL10 concentrations (via T_H2). Since IFNγ antagonises the production of IgE while IL10 stimulates and maintains it, these data indicate and explain the lack of an IgE response upon dermal exposure of mice to formalin.

Summary on skin sensitisation:

The skin-sensitising properties of formaldehyde have been addressed in numerous studies. This compound caused skin sensitisation in all of the animal studies. In particular, the LLNA in mice indicated that formaldehyde is to be considered as a strong dermal sensitiser. The most recent study conducted by Ulker *et al.* (2013) established an EC_3 value of 0.29%. By contrast, at the moment there is no experimental evidence based on animal studies that formaldehyde might similarly be capable to trigger sensitisation in the respiratory tract.

4.1.6 Dermal / percutaneous absorption

1th study: Bartnik *et al.*, 1985

Guideline:	no
Species/strain:	Rats (no data)
Membrance integrity:	Animals were clipped at a dorsal area 24 h before treatment, only rats with uninjured skin were used
Group size:	8 males and 4 females (non-occlusive), 2 males (occlusive)

Method:	*In vivo*. O/W-cream containing 0.1% formaldehyde was applied to the dorsal skin area, which had been clipped 24 h before. 200 mg cream was applied to an area of 8 cm^2 under occlusive (glass capsule) and non-occlusive (perforated class capsule) conditions. Urine and faeces were collected for 48 h and analysed for [^{14}C], and exhaled air was analysed for $^{14}CO_2$. After 48 h, rats were sacrificed and [^{14}C] was analysed in treated skin and carcasses.
Test substance:	[^{14}C]-Formaldehyde with specific activity of 47.0 mCi/mmol incorporated as tracer into non-labelled formalin (37% aqueous formaldehyde)
Batch:	no data
Purity:	Radiochemical purity of ≥95% (<2% methanol, <3% formic acid)
Test item:	O/W-cream with the following composition: 0.1% formaldehyde, 77% water, 9% fatty alcohols, 6% cosmetic oils, 3% fatty acid glycerine ester, 3% polyols, 0.5% perfume, 0.5% PHB-ester, 0.5% polyacrylates, 0.5% neutralising agents.
Dose volume:	200 mg cream.
Method of Analysis:	Determination of radioactivity ([^{14}C]-formaldehyde)
GLP:	no
Study period:	48 h

Results:
In non-occlusive experiments (n=4-8) 2.3 – 3.5% of the applied radioactivity was found in urine, 0.7 – 0.8% in faeces, 0.8 – 1.3% in expired CO_2, 1.8 – 4.1% in carcasses, and 61 - 70% in the treated skin. The total percutaneous absorption (urine, faeces, exhaled CO_2 and carcass) was 6.1-9.2%. In occlusive experiments, similar results were obtained. However, the total absorption was only 3.4%.

2nd study: Jeffcoat *et al.*, 1983

Guideline:	no
Species/strain:	Rats (Fischer 344), guinea pigs (Dunklin-Hartley), monkeys (*Cynomolgus*)
Membrane integrity:	Animals were clipped at a dorsal area 24 h before treatment, only rats with uninjured skin were used.
Group size:	8 males and 4 females (non-occlusive), 2 males (occlusive)
Method:	*In vivo*. An aqueous solution of formaldehyde was applied to a 2 cm^2 area of the shaved portion of the lower back (rats, guinea pigs), An aqueous solution of formaldehyde was applied to an 18 cm^2 shaved area on the lower back.
Test substance:	[^{14}C]-Formaldehyde dissolved in aqueous formaldehyde
Batch:	no data
Purity:	Radiochemical purity of >95%
Test item/dose volume:	Rats, guinea pigs: 0.1 mg formaldehyde in 0.010 ml solution, 11.2 mg formaldehyde in 0.040 ml solution, containing approx. 30 μCi [^{14}C]-formaldehyde each. Monkeys: 2 mg formaldehyde in 0.200 ml solution containing 590-730 μCi [^{14}C]-formaldehyde.
Method of Analysis:	Determination of radioactivity ([^{14}C]-formaldehyde)
GLP:	no
Study period:	48 h

Results:

Species	Dose/exposure conditions	Distribution of radio-activity	(% of applied dose)		Time allowed for absorption	Reference
Rat	0,1 mg (0,01 mg/µl aqueous solution, nonoccluded)	Urine: Faeces: Air traps: Carcass: Total Blood: Skin:	5.0 ± 0.6 1.5 ± 0.5 28.3 ± 2.4 22.2 ± 1.2 0.12 ± 0.01 16.2 ± 1.4	(mean ± SD of 4 male and 5 female animals)	During the first 72 h after topical administration	Jeffcoat et al., 1983
Rat	11.2 mg (0.28 mg/µl aqueous solution, nonoccluded)	Urine: Faeces: Air traps: Carcass: Total Blood: Skin:	8.3 ± 1.0 0.7 ± 0.1 22.1 ± 2.6 25.9 ± 1.9 0.13 ± 0.01 3.4 ± 0.4	(mean ± SD of 3 male and 5 female animals)	During the first 72 h after topical administration	Jeffcoat et al., 1983
Guinea pig	0.1 mg (0.01 mg/µl aqueous solution, nonoccluded)	Urine: Faeces: Air traps: Carcass: Total Blood: Skin:	4.5 ± 1.0 1.4 ± 0.2 21.4 ± 1.6 (<3% as CO_2) 27.1 ± 1.7 0.10 ± 0.02 15.6 ± 2.5	(mean ± SD of 5 male and 6 female animals)	During the first 72 h after topical administration	Jeffcoat et al., 1983
Guinea pig	11.2 mg (0.28 mg/µl aqueous solution, nonoccluded)	Urine: Faeces: Air traps: Carcass: Total Blood: Skin:	6.8 ± 1.1 1.2 ± 0.4 23.8 ± 3.1 28.4 ± 1.6 0.09 ± 0.01 3.8 ± 0.5	(mean ± SD of 5 male and 6 female animals)	During the first 72 h after topical administration	Jeffcoat et al., 1983
Cynomolgus monkey	2.0 mg (0.01 mg/µl aqueous solution, nonoccluded)	Urine: Faeces: Air traps: Carcass: Total Blood: Skin:	0.24 ± 0.1 0.20 ± 0.12 0.37 ± 0.17 n.d. 0.015 ± 0.0006 9.49 ± 3.90	(mean ± SD of 3 m animals (sex is not given)	During the first 72 h after topical administration	Jeffcoat et al., 1983

Source: "Assessment of the carcinogenicity of formaldehyde" (Schulte *et al.*, 2006)

Toxicokinetics after dermal application of [^{14}C]-formaldehyde was investigated in F344 rats, in Dunkin-Hartley guinea pigs and in *Cynomolgus* monkeys. Rodents excreted about 6.6% of the dermally-applied radioactivity in the urine over 72 h. 21 – 28% was collected in air traps. Less than 3% of the radioactivity (0.6 – 0.8% of the [^{14}C] applied) was due to [^{14}C]-CO_2. Therefore it was concluded that the major part of the air-trapped radioactivity resulted from evaporation from the skin. Rodent carcasses contained 22 – 28% of the [^{14}C] and total content in the blood was about 0.1%. Between 3.6 – 16% of [^{14}C] remained in the skin. In monkeys, 0.24% of the applied radioactivity was excreted in the urine. 0.37% of radioactivity was determined as [^{14}C]-CO_2 in air traps and about 0.015% of the radioactivity was found in total blood. 9.5% of the radioactivity remained in the skin at the site of application. Carcasses were not analysed.

Additional data from studies *in vitro*:

Loden, 1986: Dermal uptake of formaldehyde in human skin was determined *in vitro* by using a full thickness skin sample in a flow-through diffusion cell. The transcutaneous uptake of [^{14}C]-formaldehyde was measured in cryo-slices of the skin (thickness: 2 mm) after 0.5 or 15 hours of exposure. Dermal absorption rates in these experiments were 16.7 µg/cm^2/h when a 3.7% solution of formaldehyde was used and 319 µg/cm^2/h when a 37% solution of formaldehyde was used.

Summary on dermal penetration:

Animal studies applying radioactively labelled formaldehyde demonstrate penetration of this compound through the skin. The radioactivity measured in the urine and faeces of rats, guinea pigs and monkey accounted for 3 – 9%, 6 – 8%, and about 0.5% of the total doses applied, respectively.

4.1.7 Repeated dose toxicity

4.1.7.1 Subacute (up to 28 days) toxicity

4.1.7.1.1 Oral route

Study: Til *et al.*, 1988

Guideline:	OECD TG 407
Species/strain:	Rats (Wistar)
Group size:	10 males and 10 females/group, 20 males and 20 females as control group
Test substance:	Paraformaldehyde 95% plus 5% water
Batch:	no data
Purity:	no data
Dose levels:	0, 5, 25, 125 mg/kg bw
Route:	Oral (drinking water)
Exposure:	28 days
GLP:	no
Study period:	28 days, no recovery

Results:
No death occurred at 5 and 25 mg/kg bw. In males and females of the high dose group, significantly decreased food and water intake were observed. In females of the other treatment groups, the food intake was increased. In high dose males, total protein and albumin levels were significantly reduced. There was a tendency to increased urine density and decreased volume in the high dose group (both sexes). Gross pathology revealed no effects except thickening of the limiting ridge of the forestomach in males and females of the high dose group accompanied by yellowish discoloration in some animals of this group. Histopathology showed no findings except lesions of the fore- and glandular stomach consisting of focal hyperkeratosis, focal gastritis and mononuclear cell infiltrates.

Conclusions:
Formaldehyde administration via the drinking water to male and female rats for 28 days was tolerated without mortality. At 125 mg/kg bw, the thickening of the limiting ridge and hyperkeratosis of the forestomach as well as gastritis of the glandular stomach were related to treatment. Based on this observation, an oral NOAEL can be established at 25 mg/kg bw/day in male and female rats.

4.1.7.1.2 Dermal route (no guideline conform study available)

OECD, 2002: Dose finding study in mice. Dermal application of 0.1 ml of 0.1, 0.5, 1, 2, 5, and 10% formaldehyde solutions (= 0.1 – 10 mg/animal = 3 – 300 mg/kg bw) for 2 – 3 weeks. No systemic effects. Lowest doses with local effects: 0.5% (light irritation, reversible), 1% (mild irritation, starting at week 2).

4.1.7.1.3 Inhalation route (no guideline conform study available)

Studies were peer reviewed and summarised by DFG, 2000; IARC, 1995; Schulte *et al.*, 2006; ECHA 2012:

1. Wistar rats (male/female) exposed to 0, 0.3, 1 and 3 ppm for 6 h/day for 3 consecutive days. Increased cell proliferation in nasal mucosa at ≥3 ppm.

2. F344 rats and B6C3F1 mice (male) exposed to 0, 0.5, 2, 6 and 15 ppm for 6 h/day for 3 consecutive days: Increased cell proliferation in nasal mucosa at ≥6 ppm (rats) and ≥15 ppm (mice).

3. Mucociliary function in male F344 rats was disturbed starting at 2 ppm (minimal). No effect at 0.5 ppm. Exposure for 6 h/day for 14 days.

4. Nasal mucosa hypertrophy in male F344 rats started at 2 ppm. No effect at 0.5 ppm. Exposure for 4 consecutive days.

5. Rhesus monkeys (males) exposed to 6 ppm formaldehyde for 1 or 6 weeks (6 h/day, 5 days/week) developed eye irritation, epithelial hyperplasia, squamous metaplasia and inflammation in the respiratory epithelium of the nasal cavity.

4.7.1.2 Sub-chronic toxicity

4.7.1.2.1 Oral route

Study: Johannsen *et al.*, 1986

Guideline:	OECD TG 409
Species/strain:	Dogs (beagle), rats (Sprague-Dawley)
Group size:	4 males and 4 females per group (dogs), 15 males and 15 females per group (rats)
Test substance:	Paraformaldehyde diluted to 5% aqueous solution
Batch:	no data
Purity:	95%
Vehicle:	water
Dose levels:	0, 50, 75, 100 mg/kg bw (dogs), 0, 50, 100, 150 mg/kg bw = 0, 333, 666, 1000 mg/ml drinking water (rats)
Route:	Oral
Administration:	diet (dogs), drinking water (rats)
GLP:	no
Study period:	90 days

Results:
No death occurred in either species. No specific treatment-related effects were detected in haematology, clinical chemistry or urine analysis. No local effects in the gastrointestinal tract were recorded. At termination the relative and absolute organ weights were unaltered and no effects were detected in macroscopic and microscopic pathology. However, body weights were dose-dependently reduced in males and females of both species at ≥100 mg/kg bw per day (likely to be correlated to reduced water [rats] or diet [dogs] consumption).

Conclusions:
Formaldehyde orally administered via the drinking water to male and female rats or via diet to male and female dogs for 90 days was tolerated without mortality or any adverse sign of local or systemic toxicity. In particular, no effect on the stomach mucosa was observed.

4.7.1.2.2 Inhalation route

1st study: Monticello *et al.*, 1991

Guideline:	no
Species/strain:	Rats (Sprague-Dawley)
Group size:	6 males per group

Test substance:	Formaldehyde gas was generated via thermal depolymerisation of paraformaldehyde 95% (plus 5% water and 0.03% picric acid)
Batch:	no data
Purity:	95%
Vehicle:	no
Dose levels:	0, 0.7, 2, 6, 10, and 15 ppm
Route:	Inhalation
Exposure:	1, 4, 9 days and 6 weeks, 6 h/day, 5 days/week
Administration:	Whole body inhalation (8 m^3 chamber)
GLP:	no
Study period:	up to 6 weeks (42 days)

Results:
No histopathological alterations were detected at 0.7 and 2 ppm in the respiratory tract at any exposure period. At ≥6 ppm, lesions of the respiratory epithelium in the nasal cavity were observed (dose- and exposure time-dependent increases). Acute exposures (1-9 days) with 10 and 15 ppm led to vacuolar degeneration and necrosis, epithelial exfoliation, multifocal erosions and inflammatory response. Hyperplasia and metaplasia were evident after 9 days of exposure. At 6 ppm, the lesions were less severe. Six-weeks exposure at 10 and 15 ppm led to epithelial hyperplasia, squamous metaplasia, and neutrophilic infiltration. Lesions were also detected in the more posterior nasopharynx. At 6 ppm, mild lesions (hyperplasia and metaplasia) occurred only in the anterior part of the nasal cavity. Histoautoradiography revealed no alteration of the proliferation index at 0.7 and 2 ppm in any group. Significant effects were reported at a dose level of ≥ 6 ppm and exposure periods ≥ 1 day. At 6 ppm, an anterior-posterior gradient was also measured using the parameter proliferation index.

Conclusions:
The repeated inhalation of formaldehyde concentrations of up to 15 ppm for 6 h/day up to 6 weeks induced lesions of the respiratory epithelium of the nasal cavity consisting of hyperplasia and metaplasia associated with cell proliferation. NOAEL in this study: 2 ppm.

2nd study: Woutersen *et al.*, 1987

Guideline:	OECD TG 413
Species/strain:	Rats (Wistar)
Group size:	10 males and 10 females per group
Test substance:	Formaldehyde gas was generated via thermal depolymerisation of paraformaldehyde 97-99%
Batch:	no data
Purity:	97-99%
Vehicle:	no
Dose levels:	0, 1, 10, 20 ppm
Route:	Inhalation
Exposure:	13 weeks, 6 h/day, 5 days/week
Administration:	Whole body inhalation
GLP:	no
Study period:	13 weeks (90 days)

Results:
No premature death in any group. Discoloration of the fur due to inhalation procedure was observed at 10 and 20 ppm. At the highest dose (20 ppm), rats showed uncoordinated locomotion, excitation and growth retardation (both sexes). Furthermore at 20 ppm: decreased total protein and increased enzyme activity (ASAT, ALAT, and ALP) in males, epithelial thinning, keratinisation, and squamous metaplasia of the nasal olfactory and respiratory epithelium (both sexes). In males, squamous metaplasia was extended to the

larynx. At 10 ppm, focal squamous metaplasia, hyperplasia, and keratinisation were detected in the nasal respiratory epithelium of both sexes.

Conclusions:
Inhalation of formaldehyde for 13 weeks led to minor systemic effects at 20 ppm only. The predominant effects consisted of local respiratory tract irritation in the nasal cavity at 10 ppm and larynx at 20 ppm consisting of hyperplasia and metaplasia associated with cell proliferation. NOAEL in this study: 1 ppm in both sexes.

3rd study: Wilmer *et al.*, 1989

Guideline:	no
Species/strain:	Rats (Wistar)
Group size:	25 males per group
Test substance:	Formaldehyde gas was generated via thermal depolymerisation of paraformaldehyde (97-99%)
Batch:	no data
Purity:	97-99%
Vehicle:	no
Dose levels:	0, 1, 2 ppm (continuously), 0, 2, 4 ppm (intermittent)
Route:	Inhalation
Exposure:	13 weeks, 8 h/day, 5 days/week (continuously), or 8 x 30 min with 30 min non-exposure after each exposure period, 5 days/week (intermittent)
Administration:	Whole body inhalation
GLP:	no
Study period:	13 weeks (90 days)

Results:
No premature death and no clinical findings in any group. The intermittent exposure to a concentration of 4 ppm resulted in disarrangement, squamous metaplasia with and without keratinisation, and basal cell hyperplasia in the nasal cavity. No such effects were seen with continuous exposure to 2 ppm although the product of concentration and time was the same (16 ppm x h/day). This suggests that the concentration is more important than the “dose” for inducing these effects.

Conclusions:
Repeated inhalation of formaldehyde at 4 ppm led to no signs of systemic toxicity but to local respiratory tract irritation in the nasal cavity. NOAEL in this study: 2 ppm.

4th study: Maronpot *et al.*, 1986

Guideline:	OECD TG 413
Species/strain:	Mice (B6C3F1)
Group size:	10 males and 10 females per group
Test substance:	aqueous formaldehyde (aerolized by nebulisers)
Batch:	no data
Purity:	no data
Vehicle:	water
Dose levels:	0, 2, 4, 10, 20, 40 ppm
Route:	Inhalation
Exposure:	13 weeks, 6 h/day, 5 days/week
Administration:	Whole body inhalation
GLP:	no
Study period:	13 weeks (90 days)

Results:
Premature deaths occurred at 40 ppm. No clinical signs were noted up to 10 ppm. Higher concentrations led to slight dyspnoea, hunched posture up to mouth breathing and ataxia. The body weight gain was reduced at ≥10 ppm, especially in males. Dose-dependent effects occurred in the respiratory epithelium of the nasal cavity, larynx, trachea, and lung. Dose-dependent gradient of epithelial effects: nasal cavity (affected at 10 ppm), larynx/trachea (affected at 20 ppm), and bronchi (affected at 40 ppm). At 10 ppm squamous metaplasia was restricted to the anterior nasal cavity.

Conclusions:
Repeated inhalation of 40 ppm formaldehyde led to premature death in mice. At 10 ppm squamous metaplasia was found in the anterior part of the nasal cavity (both sexes). Severity and posterior extension increased dose-dependently. NOAEL in this study: 4 ppm.

4.1.7.3 Chronic (> 12 months) toxicity

4.1.7.3.1 Oral route

The potential of formaldehyde to induce systemic effects including carcinogenicity in experimental animals was addressed in several long-term studies using the oral route. However, these studies were conducted in the 1980s and none of them is in full compliance to current standard requirements on such studies.

Summary (taken from Schulte *et al.*, 2006):

Study design	Routinely performed histopathology on following organs/tissues in addition to macroscopic abnormalies:	Local toxic effects in the gastro-intestinal tract	Indications of non-specific or systemic toxicity	Hemopoietic neoplasias (HPN) (total)	Other tumor types	Remark	Reference
Groups of 70 male and female Wistar rats administered to drinking water cont. formaldehyde adjusted to achieve target intakes of 0, 5, 25 and 125 mg/kg/bw/d for up to 2 years, mean doses were 0, 1.2, 15, or 82 mg/kg bw/d for males and 0, 1.8, 21 or 109 mg/kg/bw/d for females, selected animals (10/sex/group) killed after 12 or 18 months of treatment, all survivors killed in week 105. Doses in treatment groups = average concentration of 0, 20, 260 or 1900 mg/l.	Extensive histopathology in rats of high dose + control groups: more than 30 organ/tissue samples (except thymus) All rats in low + mid dose groups: only liver, lungs, stomach, nose and, only for animals killed at week 105, adrenals, kidneys, spleen, testes thyroid, ovaries, pituitary, mammary glands.	High dose: Hyperplasia of limiting ridge, focal ulceration forestomach chronic atrophic gastritis, ulceration glandular hyperplasia	No effects on general health & survival high dose: ↓ food uptake ↓ body weight ↓ water uptake (-40%)	No treatment-related tumors	No treatment-related tumors	Higher incidence for papillary necrosis in high dose males and females, possibly related to reduced water consumption and reduced urine production	Til et al., 1989
Groups of 20 male and female Wistar rats administered drinking water containing 0, 0.02%, 0.1% or 0.5% (0, 200, 1000 or 5000 mg/l) formaldehyde for 24 months, 6 male and female rats of these groups were sacrificed after 12 or 18 months of treatment. Study ended at 24 months.	Brain, heart, lung, liver, kidney, spleen, adrenal, testis, ovary, pituitary, thyroid, stomach, small and large intestine, pancreas, uterus, lymph nodes	5000 mg/l: Erosion, ulceration fore-/glandular stomach, hyperplasia limiting ridge	5000 mg/l: Poor general health condition, sign. ↓ survival, ↓ body weight gain, ↓ water uptake ↓ diet intake	No treatment-related increase in tumors	No treatment-related increase in tumors	Small group size; limited organ spectrum, no details reported except for non-neoplastic lesions in the stomach after 12 months	Tobe et al., 1989

In the studies of Til *et al.* (1989) and Tobe *et al.* (1989), no treatment-related tumors were found upon chronic oral exposure against formaldehyde at levels up to 125 mg/kg bw/day and 300 mg/kg bw/day, respectively.
Tobe *et al.* (1989) reported at the high dose level (300 mg/kg bw/day) in both sexes a 50% decrease in food and water intake, a reduced body weight gain, and an increased mortality (approximately 50% after 12 months). Here, forestomach squamous cell hyperplasia and hyperkeratosis was observed as well as erosions and/or ulcer. One male and one female of the mid dose group (50 mg/kg bw/day) revealed with similar lesions. NOAEL in this study: 10 mg/kg bw/day.

4.1.7.3.2 Dermal route (no guideline conform study available)

Iversen, 1986: Application of 0.2 ml of 1 or 10% formaldehyde twice weekly for 60 weeks in hairless mice. Slight hyperplasia of the epidermis at 10%, no visible alterations of skin in the 1% group (= NOAEL)

4.1.7.3.3 Inhalation route

1st study: Rusch *et al.*, 1983

Guideline:	no
Species/strain:	Rats (Fischer 344), Syrian golden hamsters, Rhesus monkeys (*Cynomolgus*)
Group size:	20 males and 20 females per group (rats), 10 males and 10 females per group (hamsters), 6 males per group (monkeys)
Test substance:	5% aqueous formaldehyde
Batch:	no data
Purity:	no data
Vehicle:	water
Dose levels:	0, 0.2, 1, 3 ppm
Route:	Inhalation
Exposure:	22 h/day, 7 days/week, 26 weeks
Administration:	Whole body inhalation (exposure chamber of 7.2 m^3)
GLP:	no
Study period:	26 weeks

Results:
Rats: No premature death no clinical findings were reported. The body weight gain in males at the highest concentration of 3 ppm was slightly reduced. Gross pathology revealed no treatment-related changes at any concentration. At 3 ppm an increased incidence in squamous metaplasia and hyperplasia at the middle section level of the nasoturbinates were observed. No effects were detected in other tissues of the respiratory tract.
Hamsters: No treatment-related death. Some animals with slight and dose-dependent increases in nasal discharge, lacrimation, and rales starting at 0.2 ppm. No further treatment-related effects were noticed the respiratory tract.
Monkeys: No treatment-related death. No treatment-related effects at 0.2 and 1 ppm. At 3 ppm clinical symptoms (hoarseness, congestion, nasal discharge) were reported. In addition, squamous metaplasia and hyperplasia were observed in nasoturbinates of all animals (6/6), but not in other parts of the respiratory tract. In 1 out of 6 animals the same lesions in the nasal cavity were observable already at 1 ppm.

Conclusions:
The repeated inhalation of formaldehyde up to 3 ppm for 26 weeks induced only local effects in the epithelia of the nasal cavity of rats and monkeys. NOAEL in this study: 1 ppm (rats) and 0.2 ppm (monkeys). In hamsters, essentially no relevant effects were observed at 3 ppm under these conditions.

2nd study: Monticello *et al.*, 1996

Guideline:	no
Species/strain:	Rats (Fischer 344)
Group size:	90-147 males per group
Test substance:	Formaldehyde gas was generated via thermal depolymerisation of paraformaldehyde
Batch:	no data

Purity: no data
Vehicle: no
Dose levels: 0, 0.7, 2, 6, 10, 15 ppm
Route: Inhalation
Exposure: 6 h/day, 5 days/week, 24 months
Administration: Whole body inhalation (exposure chamber of 8 m^3)
GLP: no
Study period: 24 months

Results:
At 15 ppm a significant increase in the mortality rate was noted. At 0.7 or 2 ppm no changes in histopathology or cell proliferation were observable. Effects at 6 ppm: mixed cell infiltrate, squamous metaplasia, hyperplasia in the anterior part of the nasal cavity as well as minimal carcinogenic response. A strong increase in tumor incidence in the nasal cavity (mainly squamous cell carcinoma) was noted at 10 and 15 ppm. Dose-dependent effects on cell proliferation were detected only at ≥10 ppm.

Conclusions:
Long-term inhalation against formaldehyde led to lesions in the nasal cavity including tumor formation at 6 ppm. Steep increases in cell proliferation and tumor incidence occurred at ≥10 ppm. NOAEL in this study: 2 ppm.
Another study in rats (Kamata *et al.*, 1997) delivered some additional evidence that the NOAEL for the inhalation route may well below 1 ppm. The summary of this study is given in the table below (taken from Schulte *et al.*, 2006):

Study design Exposure to formaldehyde in ppm (mg/m³)	Routinely performed histopathology on following organs/tissues in addition to macroscopic abnormalities:	Local toxic effects in the respiratory tract	Tumors in the respiratory tract	Indications of non-specific or systemic toxicity	Other tumor types at distant sites	Remark	Reference
Rat							
Groups of 32 male F344 rats exposed to 0, 0.3, 2.17, 14.85 ppm (0, 0.36, 2.6, 17.8 mg/m³) on 5d/wk, 6 h/d, for 28 mo, interim sacrifices of 5 rats/groups after 12, 18, and 24 mo. Whole body exposure, study onset at age of 5 weeks.	Five cross sections of the nose; pituitary, thyroid, trachea, esophagus, stomach, small and large intestine, prostate gland, urinary bladder, muscle, femur, sciatic nerve, spinal cord, mesenteric lymph nodes	**Nasal cavity** ≥2.17 ppm: Squamous metaplasia, epithelial hyperplasia 14.85 ppm: Hyperkeratosis, papillary hyperplasia	**Nasal cavity:** 0 %, 0 %, 0 %, **53 %** (all nasal tumors) 15 ppm: 3 benign (squamous cell papilloma) and 14 malignant (13 SCC, 1 sarcoma) tumors	14.85 ppm: Significant ↑ in mortality, significant ↓ in body weight and food consumption	No data	Small number of animals per group, only one sex tested. Survivors at 28 mo: 0 ppm: 9, 0.3 ppm: 11, 2.17 ppm: 7, 14.85 ppm:0. Incomplete list of organs. Insufficient data to evaluate systemic toxicity/carcinogenicity	Kamata et al., 1997

At dose levels above 1 ppm the severity of lesions in the respiratory epithelium were dose-dependent. Not only the anterior part of the nasal cavity but also more proximal parts of the upper respiratory tract were affected with increasing formaldehyde concentrations, e.g., olfactory epithelium, larynx, trachea, and bronchus. One study in rats revealed that the concentration rather than the "total dose" might responsible for the effects observed at LOAEL levels (Wilmer *et al.*, 1989). At high doses (≥10 ppm) lesions in the nasal cavity of rats did not reverse anymore (Feron *et al.*, 1988).

Summary on repeated dose toxicity:

Oral route:
The main effects on rats are local lesions of the forestomach and the glandular stomach starting at about 50 mg/kg bw/day. A NOAEL was established at 10 mg/kg bw/day in the most sensitive study (Tobe *et al.*, 1989).

Dermal route:
There are no reliable studies available on repeated dose toxicity in skin. In mice only local skin effects and no systemic toxicity were induced after topical application of 0.2 ml 10% formaldehyde for 60 weeks, twice weekly (Iversen, 1986). In another study (OECD, 2002) there is evidence for local irritation at ≥0.5% formaldehyde. Based on the data available there is no evidence that systemic effects would occur after repeated dermal application of formaldehyde (for sensitisation potential cf. 3.3.3.).

Inhalation route:
In rats, mice, and monkeys the respiratory epithelium in the nasal cavity was shown to be the most sensitive site. In rats and monkeys squamous metaplasia and hyperplasia were reported, in mice rhinitis, dysplasia and squamous metaplasia. The lowest NOAEL observed in one of the studies published is 0.3 ppm in rats (Kamata *et al.*, 1997), and 0.2 ppm in monkeys (Rusch *et al.*, 1983). In rats, the majority of studies point to 1 ppm (1.23 mg/m^3) as NOAEL though.

4.1.8 Mutagenicity / Genotoxicity

A variety of genotoxic endpoints was investigated in *in vitro* assays to assess the genotoxic /mutagenic potential of formaldehyde (IARC, 1995; IPCS, 2002). The results demonstrate that formaldehyde is genotoxic to bacteria as well as to mammalian cells in culture, including nasal epithelial cells. In mammalian cells the positive genotoxic endpoints include structural chromosomal aberrations, sister-chromatid exchanges (SCE), gene mutations, DNA strand breaks, DNA protein crosslinks (DPX) and DNA repair alterations.
A fundamental aspect in the assessment of genotoxic effects of formaldehyde is whether genotoxicity *in vivo* is limited to directly exposed tissues ("local genotoxicity"), or also present at distant-site tissues ("systemic genotoxicity").

4.1.8.1 Mutagenicity / Genotoxicity *in vitro*

1. Mutagenicity in bacteria (according to OECD TG 471): Formaldehyde was demonstrated positive for inducing gene mutations in the genome of different strains of *Salmonella* in the presence or absence of S9-mix under non-cytotoxic conditions (Marnett *et al.*, 1985; Haworth *et al.*, 1983; Kamber *et al.*, 2009).

2. Mouse lymphoma cell line L5178Y mutation assay: Formaldehyde was shown to induce mutations in the mouse lymphoma cell thymidine kinase (*tk*) locus when tested with and without metabolic activation. Increases in mutagenicity were accompanied by increases in cytotoxicity (Mackere *et al.*, 1996; Blackburn *et al.*, 1991; Speit and Merck, 2002).

3. Gene mutation assay in mammalian cells (*hprt* assay): Studies on the potential of formaldehyde to induce mutations at the *hprt* locus of hamster V79 fibroblasts, hamster lung cells and human lymphoblasts led to different results. While mutagenicity could be clearly established in hamster lung cells and human lymphoblasts (Grafstroem *et al.*, 1993; Liber *et al.*, 1989), formaldehyde revealed negative in Chinese hamster V79 cells at dose ranges beyond the onset of cytotoxicity (Merck and Speit, 1998).

4. Chromosome aberration test (according to OECD TG 473): Formaldehyde revealed with a clear and concentration-dependent clastogenic activity in Chinese hamster ovary (CHO) cells or human lymphocytes at non-cytotoxic concentrations, no matter whether metabolically activated (through S9-mix), or not (Galloway *et al.*, 1985; Schmid *et al.*, 1986).

5. Micronucleus test: Formaldehyde revealed with a concentration-dependent clastogenic activity in Chinese hamster V79 cells without metabolic activation. Increases in clastogenicity were accompanied by a decrease in relative cloning efficiency as measure for cytotoxicity (Merck and Speit, 1998; Schmit and Speit, 2007; Speit *et al.*, 2007). Aneugenicity: Aneuploidy induction has not been detected in cultured myeloid progenitor cells of humans treated with 10 – 100 µM formaldehyde (Kuehner *et al.*, 2012).

6. Sister chromatid exchange (SCE) in hamster and human cells: Formaldehyde induced SCE in CHO cells both with and without metabolic activation (Galloway et al., 1985; Merck et al., 1998). This result has been confirmed by several authors in CHO cells and also extended to other cell systems such as Syrian hamster cells or human peripheral blood lymphocytes (Miyachi and Tsutsui, 2005; Schmid and Speit, 2007). Applying ex vivo human lymphocytes, no differences in the sensitivity against dose-dependent increases of SCE were observed using different study groups (male smokers, female non-smokers, children; Zeller et al., 2012).

7. Comet assay on DNA protein crosslinks (DPX) in mammalian cells: Concentration-dependent induction of DPX by formaldeyde with or without metabolic activation have been identified in a variety of mammalian including human cell cultures starting at non-cytotoxic dose levels of ≥25 µM (Merck and Speit, 1998; Quievrynet et al., 2000; Speit and Merck, 2002; Emri et al., 2004; Lui et al., 2006; Schmid and Speit, 2007; Zhao et al., 2009; Speit et al., 2010). Repeated treatment after short intervals caused an increase in DPX in Chinese hamster V79 cells but longer intervals induced a decreased effect indicating repair of DPX in these cells after 24 h (Speit et al., 2007b). Repair of DPX was also observed in human blood cells and hepatic cell lines after longer periods in fresh medium (Schmid and Speit, 2007; Zhao et al., 2009).

8. Positive results were also observed in a comet assay in human peripheral blood cells and HeLa cells (Liu et al., 2006). In the study of Liu et al. (2006), positive comet signals have been already observed starting at 5 µM in human peripheral lymphocytes and thus at concentration well below 25 µM.

Additional studies on DNA damage, DNA repair, and aneugenicity: Positive inhibition of DNA repair in human skin cells (Emri et al., 2004); Induction of altered gene expression pattern in human p53 wild-type lymphoblastoid TK6 cells (Kuehner et al., 2013);

Overall conclusion on mutagenicity / genotoxicity *in vitro*:

Formaldehyde is a highly reactive chemical with genotoxic properties. It induces various genotoxic effects; in cultured mammalian cells the induced mutations are mainly on the chromosomal level (such as structural aberrations, micronuclei), whereas there is no or only weak potential for induction of gene mutations (such as *hprt* mutations). Of the indicator endpoints induced by formaldehyde, DPX are of special importance. On the basis of the information obtained, it has to be considered that formaldehyde has *in vitro* genotoxic potential.

4.1.8.2 Mutagenicity / Genotoxicity *in vivo*

4.1.8.2.1 Local genotoxicity of formaldehyde in animals

Many investigations are published on local genotoxicity of formaldehyde in animals; almost all of them using exposure through inhalation route. Mostly these studies delivered positive findings (DNA damage) at the site of primary exposure.
One among these studies (Migliore *et al.*, 1989) was conducted with oral administration

(gavage) of 200 mg/kg bw formaldehyde to rats; increased micronuclei frequencies were found in the gastrointestinal tract. Here, the strongest genotoxic effect (micronucleus formation) was obtained in the stomach, the weakest effect in the colon. Severe local irritation was seen in parallel to genotoxicity.
Further, induction of structural chromosomal aberrations was investigated in pulmonary macrophages after inhalation exposure of formaldehyde to rats (Dallas *et al.*, 1992). Low doses of 0.5 and 3 ppm resulted in negative effects. Exposure to 15 ppm formaldehyde, however, approximately doubled the frequency of aberrant cells after 1 and 8 weeks of exposure. Conversely, a more recent GLP-conform study in rats, conducted by Neuss *et al.* (2010b), revealed no genotoxicity (i.e., micronuclei, comets, DPX) in bronchoalveolar lavage (BAL) cells at doses up to 15 ppm formaldehyde.
Several inhalation studies focussing on direct DNA effects in rats demonstrated the presence of DNA damage (DPX, comet tails) in the nasal epithelium (Lu *et al.*, 2010, 2011; Casanova *et al.*, 1989, 1994). After single 6-hrs exposures dose-dependent DPX formation was found in the nasal mucosa of rats for doses ranging from 0.3 to 10.0 ppm (Casanova *et al.*, 1989).

DNA binding study in the nasal mucosa of Fischer 344 rats (Casanova *et al.*, 1989)

Guideline:	no
Species/strain:	Rats (Fischer 344)
Group size:	4 males per group
Test substance:	[^{14}C]-Paraformaldehyde (formaldehyde gas after vaporizing: 13-20 mCi/mM)
Batch:	/
Purity:	/
Dose levels:	0, 0.3, 0.7, 2, 6, 10 ppm
Exposure:	6 h once
Route:	Inhalation (nose only)
GLP:	no

Results:
Nasal respiratory mucosal tissue has been removed and tissues of 4 rats homogenized and combined. Analysis via HPLC and scintillation counting revealed DPX formation (covalently bound fraction) in a dose-dependent but non-linear manner. Covalent binding to DNA was already observed at 0.3 ppm (ml/m^3). Binding reached levels of about 43 pmole ^{14}C/DNA/h.

Conclusion:
Formaldehyde led dose-dependently to the formation of DPX in the nasal epithelium of rats at ≥0.3 ppm and an exposure period of 6 h.

Additional studies:
In a Rhesus monkey study (Casanova *et al*, 1991), DPX yields in the respiratory tract were analysed after 6 h of exposure to formaldehyde concentrations of 0.7, 2 or 6.0 ppm. Again dose-dependent effects were found; the induction of DPX decreased with the distance (from middle turbinates to major bronchi). Based on these data a model was developed for the prediction of DPX yields in nasal mucosa of different species. Besides anatomy, main parameters were breathing volume and quantity of nasal mucosa DNA. This model was found in line with experimental data obtained for rats and monkeys. The model further suggests that DPX yields in man might be lower than in monkeys, and in monkeys much lower than in rats.
In a 1994 rat study of Casanova *et al.*, again DPX formation was seen dose-dependently for all formaldehyde concentrations investigated, ranging from 0.7 to 15.0 ppm. Here it could be shown that genotoxic effects were approximately 6 times higher in lateral mucosa cells (high tumor site) compared to cells in the medial and posterior meatus (low tumor sites). At low doses, single formaldehyde exposure for 3 h resulted in the same effects as 12-week exposures; there was no accumulation of DPX during longer exposure periods. Based on

these findings Casanova *et al.* (1994) calculated DPX yields for low dose exposures: 0.065 pmole/mg DNA/h at 0.1 ppm; 0.35 pmole/mg DNA/h at 0.5 ppm; 0.76 pmole/mg DNA/h at 1.0 ppm.

4.1.8.2.2 Systemic genotoxicity of formaldehyde in animals

There is a rather small number of studies available which investigate systemic genotoxicity of formaldehyde in experimental animals. These studies were conducted with rats or mice which were exposed to formaldehyde by inhalation, by gavage, or by intraperitoneal injection. In most of these studies inhalation was used as route of exposure, peripheral blood cells (reticulocytes, lymphocytes) and bone marrow cells served as target cells. Besides others, Speit *et al.* (2009) demonstrated the absence of DNA damage (micronuclei formation, SCE, DPX) at sites remote to the portal of entry in rats at doses of up to 15 ppm. This study was conducted under GLP conditions. The majority of studies performed in rodents, predominantly in rats, are negative (Morita *et al.*, 1997; Migliore *et al.*, 1989; Neuss *et al.*, 2010b; Speit *et al.*, 2009; Kligerman *et al.*, 1984; Casanova *et al.*, 1989, 1994). In monkeys (*Cynomolgus*) no DNA adducts or miRNA expression alterations could be found at non-target tissues either (Moeller *et al.*, 2011; Rager *et al.*, 2013). However, these studies are contrasted mainly by two inhalation studies in mice that focused on the formation of DPX in liver cells (Zhao *et al.*, 2009) or in bone marrow cells and other tissues in mice (Ye *et al.*, 2013). Zhao and coworkers demonstrated induction of significant levels of DPX in the liver of mice at an inhalation dose of 0.8 ppm for 72 h. However, they also showed that DPX numbers turned back to control levels within 12 h after treatment. The recent study performed by Ye and coworkers demonstrated dose-dependent, mainly non-linear increases in DPX levels in bone marrow, liver, spleen and testes of BALB/c mice exposed to 0.5, 1.0 and 3.0 mg/m^3 (0.4, 0.8 and 2.5 ppm) formaldehyde by nose-only inhalation for 7 days (8 h/day), with a threshold at the lowest concentration applied (i.e., 0.5 mg/m^3). Besides DPX they also looked into the levels of markers of oxidative stress (GSH, ROS, MDA) which were found altered in line with the genotoxicity observed. Among all organs tested the molecular markers under focus were found most strongly affected in the bone marrow. The SCCS notes that (i) a micronucleus test in CD-1 mice exposed to a sub-lethal oral dose of 200 mg/kg bw was negative (Morita *et al.*, 1997) and (ii) mice while more sensitive to airways irritation than rats, can reduce respiration during high inhalation exposure to irritants. Therefore, it is not clear whether reduced respiration of mice at highly irritant concentrations of 1.0 or 3.0 mg/m^3 for up to 8 hours per day may change endogenous metabolism in a way (e.g., hypoxia) that ROS or DPX formation may be favoured in peripheral blood or organs remote from the inhalation exposure site and thus may be artificial under these exposure conditions.
Whereas the majority of oral or inhalation studies with rats and monkeys did not show systemic genotoxicity or mutagenicity, there is currently uncertainty of whether or not formaldehyde can express its genotoxicity systemically in mice under certain circumstances.

4.1.8.2.3 Local genotoxicity of formaldehyde in humans

In all studies on local genotoxicity in nasal or buccal epithelia of humans formaldehyde exposure was by inhalation and micronuclei levels were used as genotoxic endpoint. In the clear majority of studies positive results were reported (Ballarian *et al.*, 1992; Titenko-Holland *et al.*, 1996; Burgaz *et al.*, 2001; Viegas *et al.*, 2010). However, micronucleus assays with nasal mucosa and buccal (exfoliated) cells are no established routine. Usually, repeated analyses show strong variations in micronucleus frequencies of the same individuals. Therefore, the results obtained require cautious interpretation.
Both of the newer studies performed under strictly controlled conditions were negative in terms of micronucleus induction at 4 h inhalation per day, 5 days per week, against up to 0.5 ppm formaldehyde (Speit *et al.*, 2007a; Zeller *et al.*, 2011). For instance, in the study of Speit *et al.* (2007a) 21 volunteers (10 women, 11 men) were exposed under strictly controlled conditions to formaldehyde vapors for 4 h per day over a period of 10 working

days. The concentration varied between constant 0.15 – 0.5 ppm, at 0.5 ppm with 4 peaks of 1.0 ppm for 15 min each. Buccal smears were sampled 1 week before start of the exposure period, immediately before the exposure period, immediately after the exposure period and 7, 14, and 21 days thereafter. At each data point 2000 cells were analysed for micronuclei. No significant effect was detected. Only a slight (not statistically significant) increase was found immediately after the exposure period. The authors concluded that formaldehyde exposures of up to 1 ppm and a cumulative exposure of 13.5 ppm x h over 2 weeks did not induce micronuclei in buccal mucosa.
The local genotoxic effects of formaldehyde in humans after inhalation exposure in the micronucleus test with exfoliated nasal or buccal epithelial cells were reviewed by Speit *et al.* in 2006. The authors overall evaluation provided some evidence for a dose-dependent increase in micronuclei frequencies in nasal and/or buccal cells after inhalation exposure. However, methodological shortcomings and limited documentation were found in most studies conducted thus far. Nevertheless, the data published were recommended to be taken as an indication that formaldehyde can express its genotoxicity at the site of first contact in humans. Yet, quantitative data on exposure and on micronucleus frequencies were not sufficiently reliable to derive further details on the dose-effect relationship.

1.1.8.2.4 Systemic genotoxicity of formaldehyde in humans

In all studies on systemic genotoxicity in humans, formaldehyde exposure occurred via inhalation. Although formaldehyde exposure hardly leads to increases of formaldehyde levels in blood (cf. section 3.3.9.), data in the literature on genotoxic effects are contradictory. So, positive and negative results were reported and a large database exists on the systemic genotoxicity of formaldehyde in humans (main target investigated: peripheral blood lymphocytes).
For micronuclei induction, positive results were consistently reported in several studies (Orsiere *et al.*, 2006, Costa *et al.*, 2008). Viegas *et al.* (2010) detected an increase in micronuclei frequency in laboratory workers but not in industrial workers, although the latter group was supposedly exposed to 5-fold higher peak concentrations. On the contrary, two recent studies did not observe such an effect. Despite exposure has been clearly confirmed in the high-level exposure group, no increases in micronuclei counts were observed by Pala *et al.* (2008). Finally, Zeller *et al.* (2011) detected no genotoxicity in peripheral blood of volunteers exposed under controlled conditions. A recent re-evaluation of slides from a published study in formaldehyde-exposed workers (Ladeira *et al.*, 2011) revealed strong variations in micronuclei measurements by different (but experienced) scorers (Speit *et al.*, 2012) and thus low/limited reliability of comparisons between the exposed and the control group.
In 2006, data on systemic genotoxicity in humans have been evaluated by Schulte *et al.* (2006). The authors concluded that due to the contradictory results, consideration of the quality of the methodology applied is of special importance. To this end, investigations were divided into 3 categories: (i) no relevant restrictions in reliability (apart from lack of GLP confirmity); (ii) not fully reliable; (iii) not sufficiently reliable, cannot be adequately assessed. In most of the studies exposure conditions of humans remained blurred. Furthermore, information on co-exposures and other confounding factors was limited. Therefore, the authors focussed on 4 'prospective' investigations where genotoxic endpoints were analysed in the very same individuals before and after exposure to formaldehyde. However, from the 4 prospective investigations available at this time no clear conclusions could be drawn either. The same was true for 11 retrospective investigations on chromosomal damage. In addition, Shaham *et al.* (2003) reported a 1.5-fold increase of DPX in human lymphocytes in 186 individuals after inhalation of formaldehyde. However, the effects observed were not to be correlated with the formaldehyde concentration. The authors of this study explain that no increases in the formaldehyde concentration in tissue or blood could be detected even moments after exposure. Hence doubts seem justified with regard to the reliability of these findings as well. Back in 2006, Schulte *et al.* explained that no clear evaluation can be made through balancing the data on systemic genotoxicity in

humans. In light of the contrasting results for systemic effects of formaldehyde in animals, the conclusion was drawn that there is no sufficient evidence to reject the plausible assumption that formaldehyde does not induce systemic genotoxicity in man.
Compared to the situation in 2006, evaluation of the additional but still contradictory animal data on systemic genotoxicity published since then (see above) might justify to be more cautious in the overall assessment of the systemically expressed genotoxicity of formaldehyde in humans exposed via inhalation.

Summary on mutagenicity / genotoxicity *in vivo*:

Formaldehyde is a highly reactive genotoxic chemical. In cultured mammalian cells the induced mutations are mainly on the chromosomal level (such as structural aberrations, micronuclei), whereas there is only a weak potential for induction of gene mutations. Of the indicator endpoints induced by formaldehyde, DPX represent the primary DNA lesions which can be processed into mutations. DPX were investigated in a number of studies in various cell locations and under various conditions.
From the investigations on systemic mutagenicity of formaldehyde in mammalian animals and humans, there is currently still some uncertainty about the genotoxic potential of formaldehyde to be expressed systemically in distant site tissues.
The data on local genotoxicity in humans need very cautious interpretation. Altogether it seems reasonable to conclude that formaldehyde can exhibit its genotoxic potential in directly exposed tissues in mammals including humans. However, no reliable data on dose-effect relationships can be derived in humans. The main focus is on data of local genotoxicity in the respiratory tract of mammals after inhalation exposure. It has been demonstrated that formaldehyde induces DPX in the respiratory tract of rats and monkeys. The differences in DPX yields between species and cell locations are likely due to different anatomy rather than biochemistry. In rats DPX were detected after inhalation of doses as low as 0.3 ppm (= $LOEC_{Rat}$) mainly in the lateral meatuses (Casanova *et al.*, 1989). In monkeys DPX are formed predominantly in the middle turbinates starting at concentrations of 0.7 ppm (= $LOEC_{Non\text{-}human\ primate}$) formaldehyde (Casanova *et al.*, 1991). No LOEC can be derived for humans.
Unlike for other toxicological effects induced by formaldehyde, for DPX formation at local, directly exposed sites no concentration without an effect could be derived. For doses up to 2 ppm there seems to be a linear dose-effect relationship for DPX induction whereas at higher doses other factors (such as cytotoxicity) have strong influence on the DPX yield resulting in non-linearity of the dose-effect relationship. Based on their extensive investigation on formaldehyde-induced DPX, Casanova *et al.* (1991) developed a model according to which DPX yields in humans are lower than in monkeys, and in monkeys much lower than in rats. For rat nasal mucosa cells, the following DPX yields were calculated for 1-hr low dose exposures (Casanova *et al.*, 1994): 0.065 pmol/mg DNA at 0.1 ppm; 0.35 pmol/mg DNA at 0.5 ppm; and 0.76 pmol/mg DNA at 1.0 ppm.
In several *in vitro* studies DPX induction was analysed in parallel to mutation endpoints. From these data a close relationship between DPX and mutations can be uncovered. This is supported by the mechanistic model that crosslinks act as bulky helix-distorting adducts thereby impairing DNA replication and thus leading to DNA strand breaks and chromosomal aberrations. Hence, formation of DPX after formaldehyde exposure has to be considered as pre-mutagenic event.
The majority of *in vivo* studies, one of them a GLP study with high inhalation concentrations of formaldehyde (Speit *et al.*, 2009), provided no evidence of systemic genotoxicity. In addition, the high local reactivity of formaldehyde with biomolecules and the level of formaldehyde formed endogenously by carbon-1 metabolism should be taken into account; Since it is therefore difficult to reach sufficiently high systemic levels of exogenous formaldehyde above the endogenous levels, potentially occurring systemic genotoxic effects triggered by exogenous formaldehyde are difficult to be distinguished from the background due to methodological reasons.

SCCS conclusion on mutagenicity / genotoxicity

Formaldehyde is genotoxic and mutagenic *in vitro* as well as *in vivo* at local sites of exposure, both in animals and humans. Oral studies in experimental animals at high doses did not show systemic genotoxicity or mutagenicity. For the inhalation route, however, the situation is less clear: Whereas the majority of studies with rats and monkeys were negative, there is currently uncertainty of whether or not formaldehyde can express its genotoxicity systemically in mice under certain circumstances.[1]

[1] SCCS is aware of IARC 2012 stating that the possibility of a mutagenic effect of formaldehyde on circulating lymphocytes or local lymphatic tissue cannot be excluded.

4.1.9 Carcinogenicity

The section 3.3.7 has been compiled based on review articles that evaluated the huge database available on the issue of formaldehyde-induced carcinogenicity in animals and man (e.g., IPCS, 2002; OECD, 2002; Schulte *et al.*, 2006; IARC, 2006, 2012; RAC, 2012).

4.1.9.1 Animals

4.1.9.1.1 Oral route

The potential of formaldehyde to induce systemic carcinogenicity in experimental animals via oral exposure was examined in several long-term studies in the 1980s (see tables below). Although none of them were fully compliant with current standard requirements, the study of Til *et al.* (1989) was considered most valid (Schulte *et al.*, 2006).

Crucial Study: Til *et al.*, 1989

Guideline:	comparable to OECD TG 453
Species/strain:	Rats (Wistar)
Group size:	70 males and 70 females per group, plus satellite animals of 10 each per sex/group
Test substance:	Paraformaldehyde (95% plus 5% water)
Batch:	no data
Purity:	95%
Dose levels:	0, 5, 25, 125 mg/kg bw/day (mean doses see table below)
Route:	Oral (drinking water)
Exposure:	up to 105 weeks
GLP:	no
Study period:	2 yrs

Results:

No death occurred and no clinical symptoms were recorded with exception of treatment-related discoloration at 25 and 125 mg/kg bw/day. Food and water consumption and thus body weight were decreased in the high dose group in males and females. Pathological alterations in the kidney (e.g., renal papillary necrosis) detected at highest dose in both sexes might be due to reduced water intake. Treatment related lesions were detected in the forestomach (focal papillary epithelial hyperplasia, ulceration and hyperkeratosis) and the glandular stomach (chronic atrophic gastritis, ulceration and hyperplasia) of males and females in the high dose group. No gastric tumors were induced. This study did not provide any evidence of carcinogenicity in rats after long-term oral administration of formaldehyde.

Conclusions:

Formaldehyde administration via drinking water to male and female rats for up to two years

was without mortality and carcinogenicity. The long-term exposure induced local effects in the stomach likely due to the irritative potential of formaldehyde. NOAEL in this study: 15 and 21 mg/kg bw/day (mean doses) in males and females, respectively.

Summary of systemic carcinogenesis of formaldehyde in animals, oral route (taken from Schulte *et al.*, 2006):

Study design	Routinely performed histopathology on following organs/tissues in addition to macroscopic abnormalies:	Local toxic effects in the gastro-intestinal tract	Indications of non-specific or systemic toxicity	Hemopoietic neoplasias (HPN) (total)	Other tumor types	Remark	Reference
Main study: Groups of 50 male and female Sprague-Dawley rats administered drinking water cont. 0, 10, 50, 100, 500, 1000 or 1500 mg/l formaldehyde for 104 weeks, groups of 100 males and females as water control groups, study end = spontaneous deaths	All groups: Brain, hypophysis, Zymbal glands, salivary glands, harderian glands, head and face bones, oral and nasal cavities, tongue, thymus, mediastinal lymph nodes, lung, heart, diaphragm, liver, spleen, pancreas, kidneys, adrenal glands, oesophagus, stomach, intestine (4 levels), bladder, prostate, uterus, vagina, gonads, interscapular fat pad, subcutaneous and mesenteric lymph nodes, sternum, femur	No data	None, no effect on survival and body weight 1500 mg/l: Water consumption –30%	Significantly dose-related increases in males and females ≥50 mg/l (see **Table 14**); the majority of them were lymphoblastic leukemias and lymphosarcomas	1500 mg/l : ↑ Tumors in glandular stomach +4% in males vs. 0% in controls; ↑ tumors in intestine +6% in males and females vs. 0% in controls	No adjustments of the concentrations to a constant dose/kg body weight; non-neoplastic effects were not reported; No historical control incidences of HPN neoplasias reported	Soffritti et al., 1989
Additional studies: Two groups of Sprague-Dawley rats administered drinking water containing 0 ppm (20 males and females) or 1500 ppm (18 males and females), two groups of their 12-day offsprings administered to drinking water containing 0 ppm (59 males and 49 females) or 2500 ppm (36 males and 37 females) for 104 weeks, study end = spontaneous deaths	Identical to the main study	No data	None on breeders, no effect on survival and body weight. Body weight decrease in offsprings (no data presented)	Slight, non-significant increase in male and female breeders and male offsprings	2500 mg/l: Stomach tumors +6% in males and females vs. 0 % in controls; intestine neoplasias +3% in males, +16% in females vs. 0% in controls	Non-neoplastic effects were not reported. no statistical evaluation	Soffritti et al., 1989
Assumed to a re-evaluation of the main study of Soffritti et al., 1989: Groups of 50 male and female Sprague-Dawley rats administered to 0, 10, 50, 100, 500, 1000 or 1500 mg/l in drinking water, 100 males and females as control group (water), study end = spontaneous death (up to 163 weeks)	All groups: Skin, sub-cutaneous tissue, brain, pituitary gland, Zymbal glands, parotid glands, submaxillary glands, harderian glands, cranium (with oral and nasal cavities, external and internal ear ducts, (5 sections), tongue, thyroid, parathyroid, pharynx, larynx, thymus, mediastinal lymph nodes, trachea, lung and mainstem bronchi, heart, diaphram, liver, spleen, pancreas, kidneys, adrenal glands, oesophagus, stomach (fore and glandular), intestine (four levels), urinary bladder, prostate, gonads, inter-scapular fat pad, sub-cutaneous and mesenteric lymph nodes	No treatment-related non-neoplastic lesions observed (no detailed data reported)	No effect on survival, feed consumption, body weight reduced water uptake at ≥500 mg/l	Significant ↑ in the number of animals with HPN at ≥100 mg/l for males and ≥1000 mg/l for females, non-sign. Increases in males at 50 mg/kg & females 500 mg/l (see **Table 14**)	1500 mg/l: ↑ Tumors in glandular stomach (14% of males vs. 0% in control); ↑ tumors in intestine (10% of males, 6% in females vs. 0% in control)	Doses were not corrected for reduced water uptakes. No dose-dependency for increase in tumors in males at 100, 500, 1000 mg/l and in females at 50, 100, 500 mg/l. Rates of HPN & gastrointestinal tumors nearly doubled in comparison to original evaluation	Soffritti et al., 2002a, 2002b

Groups of 70 male and female Wistar rats administered to drinking water cont. formaldehyde adjusted to achieve target intakes of 0, 5, 25 and 125 mg/kg/bw/d for up to 2 years, mean doses were 0, 1.2, 15, or 82 mg/kg bw/d for males and 0, 1.8, 21 or 109 mg/kg/bw/d for females, selected animals (10/sex/group) killed after 12 or 18 months of treatment, all survivors killed in week 105. Doses in treatment groups = average concentration of 0, 20, 260 or 1900 mg/l.	Extensive histopathology in rats of high dose + control groups: more than 30 organ/tissue samples (except thymus) All rats in low + mid dose groups: only liver, lungs, stomach, nose and, only for animals killed at week 105, adrenals, kidneys, spleen, testes thyroid, ovaries, pituitary, mammary glands.	High dose: Hyperplasia of limiting ridge, focal ulceration forestomach chronic atrophic gastritis, ulceration glandular hyperplasia	No effects on general health & survival high dose: ↓ food uptake ↓ body weight ↓ water uptake (-40%)	No treatment-related tumors	No treatment-related tumors	Higher incidence for papillary necrosis in high dose males and females, possibly related to reduced water consumption and reduced urine production	Til et al., 1989
Groups of 20 male and female Wistar rats administered drinking water containing 0, 0.02%, 0.1% or 0.5% (0, 200, 1000 or 5000 mg/l) formaldehyde for 24 months, 6 male and female rats of these groups were sacrificed after 12 or 18 months of treatment. Study ended at 24 months.	Brain, heart, lung, liver, kidney, spleen, adrenal, testis, ovary, pituitary, thyroid, stomach, small and large intestine, pancreas, uterus, lymph nodes	5000 mg/l: Erosion, ulceration fore-/glandular stomach, hyperplasia limiting ridge	5000 mg/l: Poor general health condition, sign. ↓ survival, ↓ body weight gain, ↓ water uptake ↓ diet intake	No treatment-related increase in tumors	No treatment-related increase in tumors	Small group size; limited organ spectrum, no details reported except for non-neoplastic lesions in the stomach after 12 months	Tobe et al., 1989
Groups of 10 male Wistar rats administered to drinking water with 0% or 0.5% formaldehyde for 32 weeks	Limited to the stomach and other organs in the peritoneal cavity (not specified)	5000 mg/l: Erosion, ulceration along the limiting ridge/ glandular stomach	5000 mg/l: ↓ Body weight gain,	No data	5000 mg/l: Fore-stomach papillomas 80% vs. 0% in controls	Small group size, short treatment duration, limited to stomach findings	Takahashi et al., 1986

Only Soffritti and coworkers reported increases of local tumors in the gastrointestinal tract of Sprague-Dawley rats (see table above). The rates of adenomas/adenocarcinomas in the stomach gland were increased in male rats administered to 1500 mg/l (4% in the original study and 14% after reevaluation; Soffritti *et al.*, 1989, 2002a). Tumor incidences in the intestine were 6% in males and females of the original study and raised to 10% in the reevaluation study. No other findings indicating cytotoxic or hyperplastic lesions were described. Although erosive-ulcerative lesions and hyperplasia in the forestomach and glandular stomach were observed at high doses, no tumor response was found in other rat carcinogenicity studies (Til *et al.*, 1989; Tobe *et al.*, 1989).

Soffritti and coworkers (Soffritti *et al.*, 1989, 2002a) also reported *systemic carcinogenicity* in rats which was in contrast to the results of other studies (Til *et al.*, 1989; Tobe *et al.*, 1989). They found a significant increase in haematopoietic neoplasias (including leukaemia and lymphoma) in Sprague-Dawley rats receiving formaldehyde with the drinking water at concentrations of 0, 10, 50, 500, 1000, or 1500 mg/l for 104 weeks. Animals were kept until their spontaneous death by week 160. At concentrations of 50 mg/l and above the incidences of haematopoietic neoplasias raised dose-dependently up to 22% for male rats and up to 14% for female rats versus 4% and 3% in control groups. Incidences of haematopoietic neoplasias were significantly higher than the control values for male rats at 1500 mg/l and for female rats at ≥1000 mg/l in the primary study (Soffritti *et al.* 1989). Tumor incidences increased dose-dependently for both sexes. In their reevaluation of the original study (Soffritti *et al.*, 2002a) the authors reported significant increases in tumor rates which became already evident at ≥100 mg/l in male rats, and were observed in female rats at ≥1000 mg/l. The maximum incidences of haematopoietic neoplasias reached 46% in male rats and 22% in female rats.
The statistical significance of the tumor response at high dose levels and its dose-dependent relationship would strongly support that formaldehyde was associated with systemic carcinogenicity in Sprague-Dawley rats. For interpretation of these data, however, some issues point to a limited validity: (1) The total number of rats with tumors listed by reevaluation had markedly increased without any explanation provided by the authors. Since no explanation was given, the extraordinary excess of tumors raises some concern on the validity of the study reevaluation and also on the credibility of the original study. (2) There is no information in these studies on incidences of haematopoietic neoplasias in historical controls. Other oral studies using Sprague-Dawley rats as test strain were not available and data from other oral carcinogenicity studies in different rat strains do not

confirm the findings of Soffritti *et al.* In the follow-up the relevance and reliability of these studies were questioned by several working groups (IARC, 1995, 2006, IPCS, 2002; NTP, 2010, OECD, 2002, Schulte *et al.*, 2006, ECHA, 2012a,b).

Summary on oral carcinogenicity in animals:

No evidence of carcinogenicity was observed in male or female Wistar rats receiving formaldehyde in the drinking water for two years. Several non-neoplastic lesions of the forestomach and glandular stomach were observed in animals treated with the highest doses. The lesions were characterized as squamous cell hyperplasia, hyperkeratosis and basal cell hyperplasia. Based on lesions of the forestomach and glandular stomach, the NOAEL was derived at 15 mg/kg bw/day in males and 21 mg/kg bw/day in females (Til *et al.*, 1989). Neither treatment-related systemic carcinogenic effects nor local carcinogenic effects in the gastrointestinal tract were reported (Til *et al.* 1989, Tobe *et al.* 1989).
However, in the drinking water studies presented by the group of Soffritti *et al.* increased incidences of tumors in the gastrointestinal tract of Sprague-Dawley rats were found at doses of up to 2500 mg/l. Due to the limitations of these studies and the strong contrast of the findings to the negative results of more reliable carcinogenicity studies in the Wistar rat, a firm conclusion on a potential for formaldehyde-associated induction of haematopoietic neoplasias in experimental animals cannot be drawn. The contradictory responses in the Soffritti studies rather might be attributable to strain-specific responses in the Sprague Dawley rat or to several shortcomings in the study design (e.g., different tumor types pooled, insufficient report on non-neoplastic endpoints, end of study = spontaneous deaths). Furthermore, no oral carcinogenicity studies are available in a second test species (e.g., mouse).
Altogether, the assumption that orally applied formaldehyde may have the potential to induce neoplasias in experimental animals is not substantiated by the current data available. The overall conclusion rather points to a very low potential for toxicity and to an insufficient evidence for local and systemic carcinogenicity of formaldehyde exerted via long-term oral exposure (RAC, 2012).

4.1.9.1.2 Dermal route (no guideline-conform studies available)

Studies that used the dermal application route while focussing on certain endpoints of toxicity can be summarized as follows: In skin tumor initiation/promotion studies the effects of formaldehyde alone (without a carcinogen for initiation) after repeated dermal application was tested in mice (Iversen, 1986). 16 male and 16 female hairless mice received twice weekly 200 µl of 1 or 10% aqueous formaldehyde to the skin of the back for 60 weeks. Complete autopsy and histopathology was performed in mice of the 10% group. No treatment-related lesions were detected macroscopically or microscopically at 1% formaldehyde. Slight epidermal hyperplasia was found in animals of the 10% group and a few mice revealed with small ulcers and scratches of the skin. No other treatment-related effects became apparent by histopathological examination of other organs. Most importantly, no skin tumors were observed in this study after exposure to formaldehyde alone (Iversen, 1986).
In the same study the tumor promoting effects of formaldehyde (200 µl of 10% formaldehyde topically applied twice weekly for 60 weeks, 16 males and 16 females) were studied after initiation with 7,12-dimethylbenz[*a*]anthracene (DMBA; single dermal application of 51.2 µg in acetone) in comparison to the DMBA-only control group. Here, formaldehyde did not increase the incidence of skin tumors but reduced significantly the latency time until the onset of tumors.
In addition, formaldehyde solutions in acetone/water (50:50) were tested by Krivanek *et al.*, 1983 (cited in OECD, 2002). Initially 50 µl of a 10% formaldehyde solution was administered to the skin followed by thrice weekly applications of 100 µl 0.1, 0.5, or 1% solution for 26 weeks (30 mice/dose). No skin tumor formation but minimal local irritation

of the skin was reported at concentrations of 0.5 and 1% (OECD, 2002).

Summary on dermal carcinogenicity in animals:

Altogether, only extremely limited data are available to assess the carcinogenic potential of formaldehyde in skin. Although some more specialized studies do provide evidence for local irritation, they do not point to an increased carcinogenic response upon repeated dermal application of formaldehyde. Nevertheless, in tumor initiation/promotion experiments formaldehyde has been capable of significantly reducing the latency time until the onset of tumors without increasing the overall tumor incidence. In view of the multilayered structure of mammalian skin, and its outmost protective multilayered non-viable *stratum corneum*, local carcinogenicity of formaldehyde in skin can be considered unlikely. Overall, no convincing evidence of a carcinogenic effect of formaldehyde via dermal route is available (RAC, 2012).

4.1.9.1.3 Inhalation route

According to Schulte *et al.* (2006) tumor inhalation studies demonstrated that formaldehyde is a nasal carcinogen in rodents such as rats and, occasionally, mice, but usually not in hamsters. According to the authors' overall conclusion in 2006 there was mounting evidence that formaldehyde vapour exerts its tumorgenicity at the sites of contact, but not at remote sites beyond the respiratory tract (e.g. bone marrow). Cytotoxicity is considered the initial lesion that induces increased cell replication, basal cell/ epithelial hyperplasia, squamous metaplasia and dysplasia; all of which constitute certain lesions that precede tumor development. In 2006, IARC supported a role for cytotoxicity and genotoxicity in formaldehyde-induced nasal tissue carcinogenesis. With regard to leukaemia, it was unclear at the time how this reactive compound could penetrate to the bone marrow, and no animal model of formaldehyde-induced leukaemia was available.
Carcinogenicity studies with mice, rats and hamsters exposed to formaldehyde by inhalation were again reviewed by IARC in 2012. The results extracted from the most reliable carcinogenicity studies are summarized in the table below. There have been no additional carcinogenicity studies in experimental animals reported since then.
In one inhalation study conducted in B6C3F1 mice, formaldehyde marginally increased the incidence of squamous cell carcinomas of the nasal cavity of males. The incidence of lymphomas in females exposed to 14.3 ppm (27/121) was marginally increased (p = 0.06) when compared (pair-wise) with controls (19/121) (CIIT, 1981; Kerns *et al.*, 1983a,b; Gibson, 1984). In several studies using different strains of rats treatment-related increases in tumours of the nasal cavity (squamous-cell carcinomas and papillomas, polypoid adenomas, rhabdomyosarcomas, adenocarcinomas, and mixed/combined tumours) were shown to occur (Swenberg *et al.*, 1980; CIIT, 1981; Albert *et al.*, 1982; Kerns *et al.*, 1983a,b; Gibson, 1984; Sellakumar *et al.*, 1985; Feron, *et al.*, 1988; Woutersen *et al.*, 1989; Monticello *et al.*, 1996; Kamata *et al.*, 1997). In one study (CIIT, 1981), the incidences of undifferentiated leukaemia were 12/120 (control), 17/120 (2 ppm), 16/120 (5.6 ppm) and 7/120 (14.3 ppm) in females; there was a marked decrease in survival in the animals exposed to the high dose. Based on a survival-adjusted analysis, the incidence of leukaemia in females exposed to 14.3 ppm was increased compared with controls (p = 0.0056). However, the Working Group noted that this type of leukaemia is a very common spontaneously occurring neoplasm in the F344 rat strain.

Table 3.1 Carcinogenicity studies in experimental animals exposed to formaldehyde

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Inhalation studies				
C3H mouse (unspecified) 35 wk (some for 64 wk) Horton *et al.* (1963)	0, 50, 100, 200 mg/m^3 1 h/d, 3 d/wk 42–60/group	No pulmonary tumours	NS	USP grade Due to severe toxicity, exposure to 200 mg/m^3 was discontinued after the 11th exposure. Thirty-six mice exposed to 50 mg/m^3 were exposed to 150 mg/m^3 for 29 additional wk. Basal-cell hyperplasia, squamous metaplasia and atypical hyperplasia were observed in trachea and bronchi of many exposed mice. Nasal tissues were not examined. Short period of exposure and short duration of study.
B6C3F1 mouse (M) 30 mo Kerns *et al.* (1983a, b), Gibson (1984)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 119–120/group	No increased tumour incidence Nasal cavity (malignant)[a]: 2/17 (14.3 ppm) vs 0/21 (controls) at 24 mo	 NS	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 0–1 at 18 mo; 17–21 at 24 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of mice exposed to 14.3 ppm.
B6C3F1 mouse (F) 30 mo Kerns *et al.* (1983a, b), Gibson (1984), CIIT (1981)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 120–121/group	No increased tumour incidence Lymphoma: 27/121 (14.3 ppm) vs 19/121 (controls)	 NS ($P = 0.06$)	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 19–20 at 18 mo; 26–41 at 24 mo; 9–16 at 27 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of mice exposed to 14.3 ppm.
F344 rat (M) 30 mo Swenberg *et al.* (1980), Kerns *et al.* (1983a, b), Gibson (1984)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 119–120/group	Nasal cavity (malignant)[b]: 0/118, 0/118, 1/119, 51/117* Nasal cavity (malignant)[b]: 0/118, 0/118, 0/119, 4/117 Nasal cavity (benign)[c]: 1/118, 4/118, 6/119, 4/117	*$P < 0.001$ NS NS	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 20 at 18 mo; 13–54 at 24 mo; 5–10 at 27 mo; 0–6 at 30 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of rats exposed to 14.3 ppm.
F344 rat (F) 30 mo Swenberg *et al.* (1980), Kerns *et al.* (1983a, b), Gibson (1984), CIIT (1981)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 120/group	Nasal cavity (malignant)[a]: 0/114, 0/118, 1/116, 52/115* Nasal cavity (malignant)[b]: 0/114, 0/118, 0/116, 1/115 Nasal cavity (benign)[c]: 0/114, 4/118, 0/116, 1/115 Haematopoietic tissue (spleen, F344 rat leukaemia diagnosed as undifferentiated leukaemia): 12/120, 17/120, 16/120, 7/120	*$P < 0.001$ NS NS $P = 0.0056$; Tarone-extension of the Cox test (adjustment for mortality), level of significance is $P < 0.0167$	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 19–20 at 18 mo; 14–47 at 24 mo; 0–10 at 27 mo; 0–5 at 30 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of rats exposed to 14.3 ppm.
Sprague-Dawley rat (M) Lifetime Albert *et al.* (1982); Sellakumar *et al.* (1985)	0, 14.3 ppm (0, 17.6 mg/m^3) 6 h/d, 5 d/wk 99–100/group	Nasal cavity (malignant)[a]: 0/99, 38/100 Nasal cavity (benign): 0/99, 10/100	$P \leq 0.001$ $P \leq 0.001$	A mixed carcinoma and fibrosarcoma of the nasal cavity was also present in the formaldehyde-treated group.

Wistar rat (M) 126 wk Feron et al. (1988)	0, 10, 20 ppm (0, 12.3 or 25 mg/m³) 6 h/d, 5 d/wk for 4, 8 or 13 wk 45/group/interval	Nasal cavity: 2/134, 2/132, 10/132 (denominator combines all intervals of exposure for control and treated groups); the authors considered 6/10 tumours[a] in the high-dose group as treatment-related.	NR	Purity NR Hyperplasia and metaplasia of nasal epithelium were observed in all rats exposed to formaldehyde. Authors considered most nasal-cavity tumours in the high-dose group to be related to the treatment.
Wistar rat (M) 28 mo Woutersen et al. (1989)	0, 0.1, 1, 10 ppm (0, 0.123, 1.23, and 12.3 mg/m³) 6 h/d, 5 d/wk for 3 or 28 mo 30/group (U: undamaged) or 60/group (D: damaged)	Nasal cavity (malignant)[b]: 28 mo exposure U: 0/26, 1/26, 1/26, 1/26 D: 1/54, 1/58, 0/56, 17/58* 3 mo exposure U: 0/26, 0/30, 0/29 2/26 D: 0/57, 2/57, 2/53, 2/54	*[$P < 0.001$; Fisher's exact test]	Purity NR Mucosa severely damaged by electro-coagulation during the first wk. Eight squamous-cell carcinomas from the nasolacrimal duct were excluded by the authors.
Sprague Dawley rat (F) 104 wk Holmstrom et al. (1989)	0, 12.4 ppm (0, 15.3 mg/m³) 6 h/d, 5 d/wk 16/group	Nasal cavity: 0/16, 1/16[a]	NS	Purity NR Pronounced squamous-cell metaplasia and/or dysplasia in 10/16 rats exposed to formaldehyde vs 0/15 controls. Small group-size noted.
F344 rat (M) 24 mo Monticello et al. (1996)	0, 0.69, 2.05, 6.01, 9.93, 14.96 ppm (0, 0.84, 2.4, 7.2, 12, 19 mg/m³) 6 h/d, 5 d/wk 90 or 147 (high dose group only)/group	Nasal cavity (malignant)[b]: 0/90, 0/90, 0/90, 1/90, 20/90, 69/147* Nasal cavity (benign)[c]: 0/90, 0/90, 0/90, 0/90, 5/90, 14/147[a] One nasal rhabdomyosarcoma and nasal adenocarcinoma each present in groups given 9.93 and 14.96 ppm	NR,*[$P < 0.001$] NR,[a][$P < 0.02$]	Formaldehyde vapour of paraformaldehyde Interim sacrifices at 3, 6, 12 and 18 mo (6/group).
F344 rat (M) 28 mo Kamata et al. (1997)	0, 0, 0.3, 2.17, 14.85 ppm (0, 0, 0.36, 2.6, 17.8 mg/m³) 6 h/d, 5 d/wk 32/group (one room-air control and one methanol-exposed control group)	Nasal cavity (malignant)[b]: 0/32, 0/32, 0/32, 0/32, 13/32* Nasal cavity (benign)[c]: 0/32, 0/32, 0/32, 0/32, 3/32	*$P < 0.01$ NR	Formaldehyde vapour of 37% aqueous formaldehyde solution with 10% methanol (4.2 ppm) Interim sacrifices at 12, 18 and 24 mo (5/group).
Rat (strain not specified) (F) Lifetime Yanysheva et al. (1998)	0, 0.003, 0.03, 0.3 mg/m³, 7 h/d, 5 d/wk for 12 mo Intratracheal injection of a total dose of 0, 0.02, 0.1 or 5.0 mg B[*a*]P/animal over 20 wk 50/group	Lung tumours: 24/35 (68.6%, 5.0 mg B[a]P + 0.3 mg/m³ formaldehyde) vs 8/28 (28.1%, 5.0 mg B[a]P)	$P < 0.01$	Purity NR Promotion effect
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 10 ppm (0, 12.3 mg/m³) 5 h/d, 5 d/wk 88/group (132 controls)	No tumours	-	Purity NR Hyper- and metaplastic areas were each observed in the nasal epithelium of 5% of exposed animals
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 30 ppm (0, 36.9 mg/m³) 5 h/d, 5 d/wk 50/group	No tumours	-	Purity NR
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 30 ppm (0, 36.9 mg/m³) 5 h/d, 5 d/wk Both groups subcutaneously injected wkly with 0.5 mg NDEA for 10 wk 50/group	Tracheal tumours: [~2.8 tumours/tumour-bearing animal (NDEA + formaldehyde) vs ~1.7 tumours/tumour-bearing animal (NDEA)]	$P < 0.05$	Purity NR Promotion effect

IPCS summarized in 2002 that most short- and medium-term inhalation toxicity studies have been conducted in rats, with histopathological effects (e.g., hyperplasia, squamous metaplasia, inflammation, erosion, ulceration, disarrangement) and sustained proliferative response in the nasal cavity at concentrations of 3.1 ppm (3.7 mg/m^3) and above (IPCS, 2002). Such effects were generally not observed at 1 or 2 ppm (1.2 or 2.4 mg/m^3), although there have been occasional reports of small, transient increases in epithelial cell proliferation at lower concentrations (Swenberg *et al.*, 1983; Zwart *et al.*, 1988). Most chronic inhalation toxicity studies have also been conducted in rats, with the development of histopathological effects in the nasal cavity being observed at formaldehyde concentrations of formaldehyde of 2 ppm (2.4 mg/m^3) and higher (Swenberg *et al.*, 1980; Kerns *et al.*, 1983a; Rusch *et al.*, 1983; Appelman *et al.*, 1988; Woutersen *et al.*, 1989; Monticello *et al.*, 1996). The IPCS report also refers to the hypothesis that formaldehyde-induced cytotoxicity is the initial lesion that precedes a proliferative response of the target tissue (e.g., hyper-/meta-/dysplasia). Thus, cell proliferation response is supposed to reflect the sites and the extent of cytotoxic lesions. The highest proliferation activities would be expected at sites with maximum lesions, and proliferation should be accelerated at or above cytotoxic

concentrations. As consequence, the highest tumor response should be expected at sites with highest cell proliferation activity.
Short-term inhalation of 1 ppm formaldehyde on 6 h/day on three consecutive days did not induce changes or altered mitotic activities in the nasal mucosa of Wistar rats (Cassee *et al.*, 1996), whereas significant increases in cell proliferation indices in the nasal mucosa of the nasal turbinates, maxillo-turbinates and of the lateral wall were observed with proliferating cell nuclear antigen expression at formaldehyde concentrations of 3.2 ppm. Nasal changes reported at this concentration consisted of disarrangement, focal necrosis, thickening, desquamation of degenerated cells, basal cell hyperplasia and/or increased numbers of mitotic figures in the respiratory epithelium.
The cell proliferation rates were also found significantly higher in the respiratory epithelium of F344 rats exposed to 6 ppm for 3, 6, 12, 18 or 24 months (transiently up to 3 months) and above (permanently at 10 and 15 ppm formaldehyde) (Monticello and Morgan, 1994; Monticello *et al.*, 1996). Comparing cell proliferation indices at different time points revealed that the increases for most mucosal sites were highest at 3 months and continuously drop to lower rates at 6, 12 and 18 months. After 3 months of exposure, the maximum labelling rate of proliferating cells was observed in the maxillo-turbinates (> the anterior lateral > anterior mid septum > posterior lateral meatus > posterior mid-septum), no response was seen in the area of maxillary sinus. These data have been summarized by Schulte *et al.*, 2006, as follows:

Table 12: Mean labelling indices per unit length for each nasal site in rats[§] after 3 months of inhalation* exposure to formaldehyde (Indices calculation without correction for number of cells/site; extracted from Monticello et al., 1996)

Formaldehyde concentration in ppm (mg/m³)	Anterior lateral meatus	Posterior lateral meatus	Anterior mid-septum	Posterior mid-septum	Anterior dorsal septum	Medial maxillo-turbinate	Maxillary sinus
0	10[#]	8	7	12	2	8	8
0.7 (0.84)	11	8	8	13	1	10	ND
2 (2.4)	10	11[§§]	13	13[§§]	3	11	3
6 (7.2)	16	10	4	11	4	9	ND
10 (12)	77	15	39	21	5	89	ND
15 (18)	93	60	76	52	6	115	11[§§§]

§ n=5 or 6, except
§§ n=4 and
§§§ n=3,
* 6 h/d, 5d/wk,
# No. of s-phase cells/mm basement membrane

Taking into account the total number of epithelial cells per area for the proliferative indices the authors found highest cell proliferation rates in the lateral meatus area (anterior > posterior) at concentrations of ≥10 ppm, a minimal response was seen at 6 ppm. Proliferation indices correlated well with the tumor rates at the different sites when the proliferation indices were corrected for cell population. The majority of squamous cell carcinomas were contributed to the area of lateral meatus.
In F344 rats exposed for 1 day, 4 days or 9 days or 6 weeks (6 h/d, 5 d/wk), 6 ppm was confirmed as the formaldehyde concentration inducing significantly elevated cell proliferation rates in sites of the lateral meatus and maxillo-turbinate from day 1 onwards. Corresponding histopathological findings were cell necrosis, inflammation, epithelial cell hyperplasia, and squamous metaplasia preferentially in the lateral meatus (> maxillo-turbinate > septum). Lesions extended to more posterior nasal levels and severity increased at higher concentrations and after longer duration of treatment (Monticello *et al.*, 1991).
Data on local effects on the respiratory tract after chronic inhalation exposure were reported with 2 ppm (2.4 mg/m^3) being the lowest concentration inducing purulent rhinitis, epithelial dysplasia and squamous metaplasia in the (anterior) level I of the nasal cavity of the rat. The lesions extended to more posterior levels I to III at 5.6 ppm, and were observed in all V

levels of the nasal cavity at 14.3 ppm (Kerns *et al.*, 1983a, Morgan *et al.*, 1986).
Formaldehyde vapour also induced lesions in the respiratory tract of Rhesus monkeys after 1 or 6 weeks of exposure to 6 ppm formaldehyde 5 days per week (6 h/d, Monticello *et al.*, 1989). Mild degeneration, epithelial hyperplasia, inflammation and squamous metaplasia was observed in the respiratory epithelium of the nasal cavity, the trachea, and major bronchi. After 1 week of inhalation, lesions were mainly confined to level II to III of the nasal passages where about one third to nearly the half of the areas were affected. The extension and severity of bilaterally symmetrical lesions increased when the inhalation period was 6 weeks, more than half of the area at the anterior levels II and III showed lesions and 20 – 40% of the posterior levels IV and V (including nasopharynx) were also damaged. Affected locations of induced lesions included all areas of the respiratory epithelium involving the nasal atrium, midseptum, lateral wall, floor of the inferior meatus, dorsal and ventral angles of the middle turbinate, and the medial aspect of the inferior turbinate. Lesions were most severe on the ventral and dorsal angles of the middle turbinate, no effects were seen in the maxillary sinuses. Also, erosions, epithelial hyperplasia, inflammatory cell inflammation and patchy hyperkeratosis were identified in the transitional epithelium of the nasal vestibule. In the 6-week treatment group, mild squamous metaplasia was observed in the olfactory/respiratory epithelial interface. In the area of the larynx/trachea, mild effects such as loss of cilia were seen in less than 3% after 1 week of inhalation, the portion of damaged area increased to 26% after 6 weeks of exposure and more extensive lesions consisting of loss of cilia and goblet cells, mild epithelial hyperplasia, early squamous metaplasia were observed. The authors concluded that comparing the extent of lesions and cell proliferation data at 6 ppm formaldehyde, monkeys appeared to be more sensitive than rats (Schulte *et al.*, 2006).

Summary on inhalation carcinogenicity in animals:

In rats and monkeys, formaldehyde-induced lesions and reactive cell proliferation responses were most pronounced in the transitional and respiratory epithelium of the nasal passages. The nature of lesions observed as epithelial degeneration/cell necrosis and secondary to cytotoxicity, inflammatory cell response, squamous metaplasia and epithelial hyperplasia/ dysplasia (so-called 'non/pre-neoplastic lesions'). Increased cell proliferative responses were similar in rats and in monkeys. The three-dimensional distribution of sub-sites with maximum responses were slightly different among species: In rats, the lateral meatus was the site that reacted earliest, at lowest concentration and with highest severity with cytotoxic and hyper/metaplastic changes and elevated cell replication (Monticello *et al.*, 1996, 1991, Casanova *et al.*, 1994). In monkeys exposed to 6 ppm formaldehyde for 6 weeks, the ventral and dorsal angles of the middle turbinate of the anterior nasal passages were most severely affected at more than half to the area (Monticello *et al.*, 1989). More distally, about 40% of the mucosa in the posterior level V (including the nasopharynx) was damaged, which indicated that this airway area was also highly susceptible.
Across species, there was an anterior-posterior gradient of non/pre-neoplastic lesions being milder in the posterior regions. In rats and monkeys, the distributions of non/pre-neoplastic lesions extended towards more posterior levels of the nose and in severity with treatment prolongation (Monticello *et al.*, 1989, 1991). The lowest effective concentration inducing non/pre-neoplastic lesions after chronic exposure was 2 ppm (6 h/d, 5 d/wk, up to 24 months) (Kerns *et al.*, 1983a). The lowest effective concentration from short-term exposure studies was 3.2 ppm (6 h/d, 3 d) in rats (Cassee *et al.*, 1996). In monkeys, 6 ppm (6 h/d, 5 d/wk) was the lowest effective concentration for short-term exposure during 1 or 6 weeks (Monticello *et al.*, 1989).
In rats and monkeys, the sites of mucosal lesions corresponded to the areas with increased cell proliferation. In the monkey nose, the middle turbinate exhibited the most extended lesions and the strongest response in replication rates (uncorrected for epithelial thickness). Comparing cytotoxicity and mitogenicity at cross sectional levels, the grades of effects corresponded at levels II, III and V (highly affected) and at level IV (minor changes). In rats, the sites of high cell proliferative activity were found to correspond to the mucosa

areas where most frequently the tumor growth origined from ('high tumor site'). In the rat, this was the lateral meatus (Casanova *et al.*, 1994, Monticello et al, 1996). No tumor data are available for the monkey and no comparison could be drawn.

RAC, 2012, summarized these studies as follows:

Table 25. Incidence of tumours and precursor lesions in the nasal cavity of rats following inhalation

Dose (ppm)	**0.1** [a]	**0.3** [b]	**0.7** [c]	**1** [a]	**2** [c]	**2** [b]	**2** [d]	**5.6** [d]	**6** [c]	**10** [a]	**10** [c]	**14.2** [e]	**14.3** [d]	**15** [b]	**15**
Squamous cell carcinomas (%)	0	0	0	0	0	0	0	0.8	1	4	22	38	44	41	47
Other malignant tumours* (%)	0	0	0	0	0	0	0	0	0	0	2	2	2	3	1.4
Polyps, papillomas or polypoid adenomas (%)	0	0	0	0	0	0	3	2.6	0	0	5.6	10	2	9	9.5
Signs of chronic irritation															
Epithelial cell yperplasia	-	+	-	-	-	+	-	-	-	+	+	-	+	+	+
Epithelial dysplasia	NR	NR	-	NR	NR	NR	+	+	NR	NR	NR	NR	+	NR	NR
Squamous cell metaplasia	-	+	-	-	-	+	+	+	+	+	+	+	+	NR	+
Rhinitis	-	-	-	-	-	+	+	+	NR	+	NR	-	+	+	NR
Cell infiltration	NR	-	-	NR	-	-	NR	NR	NR	NR	+	NR	NR	-	+
Edema	NR	-	-	NR	-	-	NR	NR	NR	NR	NR	NR	NR	-	NR

[a] Woutersen 1989; [b] Kamata 1997; [c] Monticello 1996; [d] Kerns 1983; [e] Sellakumar 1985; * carcinoma, carcinosarcoma, fibrosarcoma, rhabdomyosarcoma; +: sign reported as present; -; sign reported as absent; NR: not reported

Overall, the inhalation carcinogenicity of formaldehyde is well established in rats with induction of tumours at the site of contact. Formaldehyde is highly cytotoxic and irritant, and nasal tumours are observed only at doses producing chronic irritation as evidenced by the accompanying inflammatory, hyperplastic and metaplastic responses. Among species, the degree of sensitivity to nasal irritation is associated with the degree of sensitivity to nasal tumour induction. Localisation of damage to the nasal epithelium also corresponds with tumour site and distribution is attributable to regional dosimetry and/or local tissue susceptibility.

A consistent database provides evidence that regenerative cell proliferation secondary to cytotoxicity highly correlates with tumour incidence and regional distribution. Regenerative cell proliferation is observed at 10 and 15 ppm with 6 ppm being a borderline concentration (Monticello *et al.,* 1996, Casanova *et al.* 1994, Meng *et al.,* 2010). At higher doses, cytotoxicity is followed by regenerative cell proliferation. An increased rate in cell proliferation is associated with a larger probability of fixing a primary DNA lesion as a mutation and a decrease in the time available for DNA repair. Observation of hyperplastic and metaplastic changes strongly support the hypothesis of a mechanism promoted by regenerative proliferation and accompanied by an inflammatory response that may also

contribute to the genotoxicity of formaldehyde (Schulte *et al.*, 2006). A steep increase in tumour induction is therefore expected at doses exerting cytotoxicity and regenerative cell proliferation. This is also consistent with the induction of chromosomal aberrations at the site of contact at high doses (Dallas *et al.,* 1992). Experimental results and mechanistic data therefore support the existence of a threshold-type dose-response for induction of nasal tumours with regenerative cell proliferation being the predominant feature in this carcinogenic process.
On the other hand, there is no convincing evidence of a carcinogenic effect at distant sites or via other routes of exposure than inhalation.

4.1.9.2 Humans

Numerous studies looked into an association of formaldehyde exposure with cancer incidence. They consist of cohorts, case-control studies and meta-analyses. In all of these studies, human exposure was by inhalation. Cohorts report mortality or incidence of cancers in two types of exposed workers: industrial cohorts from formaldehyde production plants, resin plants or other industries using formaldehyde or professional cohorts of embalmers or anatomists/pathologists. Three large, recently-updated industrial cohorts are considered as the most informative: the NCI cohort (Beane Freeman *et al.*, 2009 and Hauptmann *et al.*, 2004), the British cohort (Coggon *et al.*, 2003) and the NIOSH cohort (Pinkerton *et al.*, 2004) include large populations and provide detailed assessments of the levels of exposure.
In the overall weight of evidence, it is considered that studies showing a statistically significant excess of risk supported by statistically significant trends with one exposure metrics provide the strongest level of evidence that the observed carcinogenic effects is related to formaldehyde exposure (IARC, 2012). In addition to the studies reporting statistically significant excess of risk, the studies with a non-statistical excess of risk but with a positive trend for exposure levels were also considered as supportive evidence.

4.1.9.2.1 Nasopharyngeal cancer

IARC concluded in 2006 that there was *sufficient evidence* for the carcinogenicity of formaldehyde, primarily based on its association with nasopharyngeal cancer. There have been only some new studies published on this association since that time:
a) Cohort studies: In the most recent follow-up of the largest cohort study from the US of industrial workers exposed to formaldehyde ("NCI cohort"), a statistically significant excess of deaths from nasopharyngeal cancer was observed in comparison with the general US population, with statistically significant exposure–response relationships for peak exposures and cumulative exposures (Hauptmann *et al.*, 2004). Based on eight cases, a significant excess mortality from nasopharyngeal cancer was observed among formaldehyde-exposed workers (SMR: 2.10; 95% CI: 1.05 – 4.21). A highly statistically significant (P_{trend} <0.001) exposure–response relationship was observed between peak-exposures to formaldehyde and risk for nasopharyngeal cancer in a Poisson regression analysis. All exposed cases were in the highest category of peak-exposure, and the relative risk was 1.83. Weaker exposure–response relationships were observed between nasopharyngeal cancer and average or cumulative exposure, and duration of exposure (P_{trend} = 0.07, 0.03 and 0.15, respectively).
In the two other large cohort studies of industrial workers, cases of nasopharyngeal cancer were fewer than expected, but the power of these studies to detect an effect on nasopharyngeal cancer was low and the deficits were small. In the first study, among British chemical workers one death was observed when 2 were expected (Coggon *et al.*, 2003); in the second study, no deaths were observed among US garment-manufacturers, where 0.96 were expected (Pinkerton *et al.*, 2004). An excess of deaths from nasopharyngeal cancer was observed in a proportionate mortality analysis of the largest US cohort of embalmers (Hayes *et al.*, 1990) and in a Danish study of proportionate cancer incidence among workers

at companies that used or manufactured formaldehyde (Hansen and Olsen, 1995). Marsh *et al.* (1996) conducted a cohort study in one of the plants considered in the NCI study (where five of the nine cases of nasopharyngeal cancer occurred). The cohort included earlier year of entry and was enumerated independently. Significantly increased mortality due to nasopharyngeal cancer was observed among formaldehyde-exposed workers compared with US and regional populations. In a recent follow-up through 2003, Marsh *et al.* (2007a) showed elevated standardised mortality ratios (SMRs) when both national and local county rates were used. In addition, when conducting a case–control study nested within the cohort and including seven deaths from nasopharyngeal cancer, the authors obtained information on employment outside the formaldehyde industry and showed that five of these workers had been employed as a silversmith. However, while there was some evidence of effect modification by activities as a silversmith (based on small numbers), confounding alone did not explain the relatively high number of deaths from nasopharyngeal cancer in this plant (Marsh *et al.*, 2007a). Two analyses have been conducted to re-analyse the data from the most recent update of the NCI cohort, with a focus on solid tumours (Hauptmann *et al.*, 2004). The first included an analysis of exposure category and SMR, as well as an analysis of plant 1, where five of nine deaths from nasopharyngeal cancer occurred, compared with all other plants in the cohort (Marsh and Youk, 2005). Using their own cutpoints of exposure, the authors concluded that their analysis lent uncertainty to the findings from the NCI cohort. In another re-analysis, the authors further controlled for the effect of plant for the peak-exposure metric and performed sensitivity analyses by imputing additional cases, which showed instability in the risk estimates (Marsh *et al.*, 2007b). The authors concluded that an interaction between plant group and exposure makes generalisation beyond plant 1 difficult.

b) Case-control studies: The relationship between nasopharyngeal cancer and exposure to formaldehyde has also been investigated in seven case–control studies, five of which found elevated risks for overall exposure to formaldehyde or in higher exposure categories (Vaughan *et al.*, 1986b; Roush *et al.*, 1987; West *et al.*, 1993; Vaughan *et al.*, 2000; Hildesheim *et al.*, 2001). One study found an elevation among women, but not men (Olsen *et al.*, 1984), and one found no evidence of an association (Armstrong *et al.*, 2000). Two case–control studies were considered as the most informative because of their size, their exposure assessment, and the evaluation of potential confounders. The first, a population-based case–control study in the US, showed a significant association for the workers whose exposure duration had been the longest (OR = 2.1; 95% CI: 1.0 – 4.5, P_{trend} = 0.07), but not for maximum exposure (P_{trend} = 0.57) (Vaughan *et al.*, 2000). When the analysis was limited to differentiated squamous-cell and epithelial “not-otherwise-specified” (NOS), there was a significant association in the highest exposure category for both duration and cumulative exposure with significant exposure-response trends (P_{trend} = 0.014 and 0.033, respectively). In the other study, an OR of 1.6 (95% CI: 0.91 – 2.9, P_{trend} = 0.08) was found in the category with the longest duration of exposure (Hildesheim *et al.*, 2001). For cumulative exposure, however, there was only a non-significant elevation in the highest exposure category and the trend test was not significant (P = 0.10).

c) Meta-analyses: A meta-analysis published in 1997 included some but not all of the above studies, and found an overall meta-relative risk for nasopharyngeal cancer of 1.3 (95% CI: 1.2 – 1.5) (Collins *et al.*, 1997). From a pooled analysis including the three recently updated industrial cohorts (Coggon *et al.*, 2003; Hauptmann *et al.*, 2004; Pinkerton *et al.*, 2004), Bosetti *et al.* (2008) reported an overall SMR of 1.33 (95% CI: 0.61 – 2.53). A recently published meta-analysis included both case–control studies (n = 6) and cohort studies (n = 7) (Bachand *et al.*, 2010). For the case–control studies, the overall OR was 1.22 (95% CI: 1.00 – 1.50), with the meta-regression OR no longer significant when limited to studies that included adjustment for socio-economic status, smoking or location. By contrast, the risk estimate for cohort studies was 0.72 (95% CI: 0.40 – 1.29), when including seven studies.

Conclusions on nasopharyngeal, pharyngeal, laryngeal and lung cancers in humans (according to RAC, 2012):

There is consistent evidence from the NCI cohort and from several case control studies that formaldehyde may induce *nasopharyngeal cancer.* The existence of a grouping of cases in plant 1 of the NCI cohort raises some doubt on potential cofounders and lowers the level of evidence but it can also be explained by the largest number of subjects exposed to high peaks in this specific plant. Evidence of a link between exposure to formaldehyde and induction of *pharyngeal cancer* (other than nasopharyngeal) is provided in case-control studies, particularly in Laforest *et al.* (2000). Data from cohorts are inconsistent and overall provide no clear evidence of an increased risk of pharyngeal cancer. Data from cohort studies provide no evidence of an increased risk of *laryngeal cancer* to support the slight increase identified in some case-control studies. The inconsistency of the results in the large industrial cohorts, the discrepancy between results in industrial and professional workers and the potential cofounders in small industrial cohorts does not allow to identify an association between formaldehyde exposure and *lung cancer.*

4.1.9.2.2 Sinonasal cancer

a) Cohort studies: An analysis of proportionate cancer incidence among industrial workers in Denmark showed an increased risk for squamous-cell carcinomas (Hansen and Olsen, 1995, 1996). No excess of mortality from sinonasal cancer was observed in three recently updated studies of industrial and garment workers in the US, and of chemical workers in the United Kingdom (Coggon *et al.*, 2003; Hauptmann *et al.*, 2004; Pinkerton *et al.*, 2004).
b) Case-control studies: The association between exposure to formaldehyde and the risk for sinonasal cancer has been evaluated in six case–control studies that primarily focused on formaldehyde (Olsen *et al.*, 1984; Hayes *et al.*, 1986; Olsen and Asnaes, 1986; Vaughan *et al.*, 1986a; Roush *et al.*, 1987; Luce *et al.*, 1993; Pesch *et al.*, 2008). Four of these six studies reported an increased risk (Olsen *et al.*, 1984; Hayes *et al.*, 1986; Vaughan *et al.*, 1986a; Luce *et al.*, 1993).
c) Meta-analyses: Four of the cohort studies contributed to a pooled analysis that collated occupational data from 12 case–control investigations (Luce *et al.*, 2002). After adjustment for known occupational confounders, this analysis showed an increased risk for adeno-carcinoma associated with high exposure (>1 ppm) to formaldehyde in both men (OR: 3.0; 95% CI: 1.5 – 5.7) and women (OR: 6.3; 95% CI: 2.0 – 19.7). An exposure-dependent response trend was observed in relation to an index of cumulative exposure. There was some evidence of an association with squamous-cell carcinoma.

Conclusions on sinonasal and oral cavity cancers in humans (according to RAC, 2012):

Evidence of a link between exposure to formaldehyde and induction of *sinonasal cancer* is provided in case-control studies. However, it is not observed in industrial or professional cohorts as the positive association in the Danish cohort (Hansen and Olsen, 1995, 1996) is not reproduced in the largest industrial cohorts. Therefore it can be concluded that there is some evidence from case-control studies and no or no significant evidence from available cohort studies. Data are considered to be insufficient to conclude on an association of formaldehyde exposure with sinonasal cancer. In addition, data from cohorts are inconsistent and no result from any reliable study attained statistical significance and data are not considered as sufficient to provide a causality relationship between formaldehyde and *cancers of the oral cavity*.

4.1.9.2.3 Cancer at distant (remote) sites

An excess of lymphohaematopoietic cancers is reported most specifically for *leukaemia*. A non-statistically significant increase was reported in two large industrial cohorts with support of positive trends for peak and average intensity (NCI cohort; Hauptmann *et al.*, 2003) and for duration and time since first exposure (NIOSH cohort; Pinkerton *et al.*, 2004).

Non-statistically significant increases in risk were reported in several professional cohorts that were supported with trend for duration in Walrath *et al.* (1984) but not in Stroup *et al.* (1986), as well as in two case-control studies.

In 2006 IARC summarized that there was strong, but not sufficient evidence for the leukaemogenic effects of formaldehyde. Since that time, an update to the NCI cohort and a nested case–control study of workers in the funeral industry have been published (Beane Freeman *et al.*, 2009; Hauptmann *et al.*, 2009), as well as three meta-analyses (Bosetti *et al.*, 2008; Zhang *et al.*, 2009; Bachand *et al.*, 2010):

According to IARC 2012, excess mortality from leukaemia has been observed consistently in studies of professional workers (i.e., embalmers, funeral parlour workers, pathologists and anatomists), with six mortality studies showing positive associations (Walrath and Fraumeni, 1983, 1984; Levine *et al.*, 1984; Stroup *et al.*, 1986; Hayes *et al.*, 1990; Hall *et al.*, 1991) and one not (Logue *et al.*, 1986). However, a weakness of the proportionate mortality studies among professionals has been the lack of exposure assessment. A recently published nested case–control study conducted among professionals in the funeral industry examined lifetime work practices and exposure in the funeral industry to develop metrics of exposure among this group, which included duration of jobs held while embalming, number of embalmings, average intensity of embalming and peak exposure (Hauptmann *et al.*, 2009). At many levels of exposure and for multiple exposure metrics positive associations were seen for deaths from lymphohaematopoietic malignancies of non-lymphoid origin (n = 48). For myeloid leukaemia (n = 34) the OR was 13.6 (95% CI: 1.6 – 119.7; P_{trend} = 0.020) for the longest duration of work in jobs with embalming. Because only one case was reported to have never embalmed, additional analyses were conducted in which those who reported to have embalmed ≤500 times were taken as the reference group, to provide a more stable estimate. Results were attenuated, but still significant (OR = 3.9; 95% CI: 1.2 – 12.5).

The findings for leukaemia in studies of professional workers appeared to be contradicted by the lack of such findings among industrial workers. However, some evidence for an excess of deaths from leukaemia has been reported in the recent updates of two of the three major cohort studies of industrial workers. Since the previous evaluation (IARC, 2006), the NCI cohort of industrial workers in the US has been updated with an additional ten years of mortality data resulting in 123 deaths from leukaemia, including 48 from myeloid leukaemia (Beane Freeman *et al.*, 2009). This update extended the mortality follow-up before 1994 that had not been previously considered. Risk estimates from follow-up through 2004 were diminished for leukaemia and myeloid leukaemia compared with the follow-up through 1994 (Hauptmann *et al.*, 2003), when both conditions had been significantly associated with increasing peak-exposure and average intensity of exposure to formaldehyde. As in the previous analysis of leukaemia, the association in the most recent update was stronger for myeloid leukaemia and peak exposure than for lymphatic leukaemia and for other metrics of exposure (Beane Freeman *et al.*, 2009). However, because the last known exposure occurred in 1980 and median follow-up was over 40 years, the authors not only examined risks at the end of follow-up in 2004, but also assessed associations over time by extending follow-up in yearly increments. Risks appeared to be highest before 1980, but only achieved statistical significance in the mid-1990s, when a sufficient number of deaths had occurred. Additional analyses with time since first exposure and time since first high peak-exposure indicated that risks were highest during the first twenty-five years. Patterns were similar, but attenuated, for average intensity of exposure; no association was observed with cumulative exposure.

Mortality from leukaemia was also found to be in excess in an update of the study of US garment workers exposed to formaldehyde (Pinkerton *et al.*, 2004). A small and statistically non-significant excess was observed for the entire cohort in comparison with rates among the general population (SMR = 1.09; 95% CI: 0.7 – 1.63). This excess was somewhat stronger for myeloid leukaemia (SMR = 1.44; 95% CI: 0.80 – 2.37), which is consistent with the findings from the study of industrial workers in the US and several of the studies of medical professionals and embalmers. The excess was also stronger among workers with a longer duration of exposure and longer follow-up, and among those who had been

employed early in the study period when exposures to formaldehyde were believed to be highest. The positive associations observed in the subgroup analyses presented in the study of US garment workers were based on a relatively small number of deaths, and were thus not statistically stable.

The updated study of British industrial workers found no excess mortality for leukaemia among all workers exposed to formaldehyde (SMR = 0.91; 95% CI: 0.62 – 1.29) or among those with the highest exposure (SMR = 0.71; 95% CI: 0.31 – 1.39) (Coggon *et al.*, 2003). The lack of positive findings in this study is difficult to reconcile with the findings from the studies of garment workers and industrial workers in the US, and with the results of studies on professionals exposed to formaldehyde. This British study is a relatively large, high-quality study with sufficiently long follow-up to have had a reasonable chance to detect an excess of deaths from leukaemia. It did not examine specifically the risk for myeloid leukaemia, which represented the strongest finding in the studies of garment workers and industrial workers in the US and in several of the studies of medical professionals and funeral workers.

Meta-analyses: A meta-analysis published in 2004 for 'ever exposure' to formaldehyde and leukaemia included eighteen studies and presented separate analyses by type of job: for industrial workers, the mRR was 0.9 (95% CI: 0.8 – 1.0); for embalmers 1.6 (95% CI: 1.2 – 2.0); and for pathologists and anatomists 1.4 (95% CI: 1.0 – 1.9), with an overall mRR of 1.1 (95% CI: 1.0 – 1.2) (Collins and Lineker, 2004). In another meta-analysis, analysis was restricted to 13 cohort or proportionate mortality studies and similar results were found, with a pooled RR based on the weighted average of the SMRs for leukaemia among industrial workers of 0.9 (95% CI: 0.75 – 1.07), based on 122 deaths, and of 1.39 (95% CI: 1.15 – 1.68) among professionals, based on 106 deaths (Bosetti *et al.*, 2008). A further meta-analysis differed from these two previous ones by excluding all proportionate mortality studies and including the most recent update of the NCI cohort (Bachand *et al.*, 2010). For overall leukaemia, a risk estimate of 1.05 (95% CI: 0.93 – 1.20) was calculated for 'ever exposure', based on 15 studies with the use of a fixed-effect model. For myeloid leukaemia, the calculated mRR was 1.09 (95% CI: 0.84 – 1.40, based on three studies) and for lymphatic leukaemia the mRR was 1.11 (95% CI: 0.81 – 1.52, based on two studies). Zhang *et al.* (2009) published a meta-analysis that included 15 cohort or case–control studies. The authors selected only studies where it was clear that the workers had been exposed to formaldehyde. In contrast to the other meta-analyses, this one used one exposure metric from each study and considered the highest exposure category for calculating the mRR. For leukaemia, the mRR was 1.54 (95% CI: 1.18 – 2.00). In addition, a separate analysis of myeloid leukaemia found an mRR of 1.90 (95% CI: 1.31 – 2.76).

Conclusions on cancers at distant sites in humans (according to RAC, 2012):

Overall, some positive observations have emerged in industrial populations but meta-analyses generally show a discrepancy in the results between industrial and professional populations in which several studies indicate an increased risk of leukaemia and especially myeloid leukaemia. Therefore, it is considered that available data *do not provide causal evidence for formaldehyde as the aetiological factor (of leukaemia)* as a bias specific to professionals cannot be ruled out. Isolated results across studies suggest also an elevated risk of cancers at other sites such as *stomach, rectum, pancreas, prostate, breast, colon, oesophagus, thyroid,* etc. However, these results were highly inconsistent for stomach, brain, colon, pancreas and prostate with excess of cancers limited to either industrial workers or professionals and not identified in the largest industrial cohorts.

Carcinogenicity in humans (overall conclusion drawn by RAC, 2012)

The biological plausibility of the induction of nasopharyngeal carcinomas in humans exposed to formaldehyde highly supports the consistent epidemiological evidence obtained from the NCI cohort and from several case-control studies. The data support a causal relationship

between formaldehyde exposure and induction of nasopharyngeal cancers and corresponds to a sufficient evidence of carcinogenicity in humans. Conversely, in absence of convincing evidence for a biologically plausible mechanism and considering the discrepancy of results in epidemiological studies, a causal relationship between formaldehyde exposure and induction of myeloid leukaemia cannot be concluded.

Carcinogenicity in humans (overall conclusion drawn by IARC, 2012)

The Working Group noted one industrial cohort study with both a strong overall association between exposure to formaldehyde and nasopharyngeal cancer, and the most elevated risks in the highest exposure category. Positive associations were also observed in many of the case–control studies, in particular those of larger size and higher-quality exposure assessment. It is concluded that occupational exposure to formaldehyde causes nasopharyngeal cancer in humans. Elevated risks of leukaemia have been consistently observed in proportionate mortality studies of professionals exposed to formaldehyde (i.e., embalmers, workers in the funeral industry, pathologists and anatomists). Results from a nested case–control study of workers in the funeral industry showed elevated risks for many measures of exposure, which are strongest for myeloid leukaemia. In two of the three large industrial cohort studies positive associations were observed for leukaemia, which were somewhat stronger for myeloid leukaemia. It is difficult to reconcile the lack of association observed in the third industrial cohort study with the overall positive associations in the others. However, there seems to be no strong evidence that confounding or bias explains the positive associations seen in multiple settings. On balance, the Working Group concluded that the epidemiologic evidence shows that occupational exposure to formaldehyde causes leukaemia.

4.1.10 Reproductive and developmental toxicity

4.1.10.1 Animals (Summary)

Reproductive and developmental toxicity of formaldehyde in animals has recently been summarized by Nielsen *et al.* (2013). The following paragraphs provide brief digest of their evaluation.

In a study where females were exposed to 5, 10, 20 or 40 ppm formaldehyde for 6 h/day from gestational day 6–20 (Saillenfait *et al.*, 1989) decreased body weight gain was observed in the dams only at the highest exposure level (40 ppm), but no teratogenic effect at this dose. Another developmental toxicity study in female rats (exposed to 2, 5 or 10 ppm formaldehyde for 6 h/day from gestational day 6 to 15) showed a NOAEL for maternal toxicity (reduced food consumption) at 5 ppm but no teratogenic effect at 10 ppm (Martin, 1990).

In a more recent study (Carmines & Rajendran, 2008) pregnant rats exposed to the mixture of 1 mg/m^3 formaldehyde (0.8 ppm), 41 mg/m^3 acetaldehyde (23 ppm) and 4 mg/m^3 acrolein (1.7 ppm) by inhalation revealed without any adverse effect on the dams or its offspring. Although embryo culture studies clearly demonstrated the *ex vivo* embryotoxicity of formaldehyde (Hansen *et al.,* 2005), such effects have never been observed in inhalation studies, thus indicating that formaldehyde does not reach the embryo by inhalation.

In a study where adult male rats were exposed for 8 h/day, 5 days/week for 4 and 13 weeks, body weight gain and testis weights were significantly reduced in the low (10 ppm) and high (20 ppm) formaldehyde groups compared to controls (Ozen *et al.*, 2002). Food and water consumption were also decreased in the treatment groups. Formaldehyde at these concentrations led to significant reductions of serum testosterone concentrations (down to 60 and 35%) and mean seminiferous tubule diameters (down to 91 and 90%) compared to controls. In another (2-week) study, male rats were exposed to 10 mg/m^3 formaldehyde (8 ppm) for 12 h/day and revealed with decreased testicular weight, atrophy

of the seminiferous tubules, decreases in spermatogenic cells, seminiferous epithelial cell disintegration, interstitial tissue oedema with vascular dilatation and hyperemia. Further, luminal azoospermia, decreased epididymal sperm counts, and increases in abnormal sperm counts were observed in the formaldehyde treatment group.
Recently, Zhou *et al.* (2011) looked into the effects of 0.5 and 2.46 mg/m^3 (0.4 and 2 ppm) formaldehyde given for 8 h/day. In this study no differences were observed in serum testosterone concentrations, testicular and epididymal weights, nor in epididymal tubular diameters. In the high-exposure group, however, atrophy of the testicular seminiferous tubules, decreased spermatogenic cells and oligozoospermia were observed. Additionally, testicular seminiferous tubular diameters and epididymal sperm counts were significantly decreased, while the epididymal percentage of abnormal sperms were significantly increased. Thus, this study offered a NOAEL of 0.4 ppm, a level where neither sensory irritation nor decreased respiratory minute volume was observed in the rats. Hence no effect was observable in male rats in the absence of sensory irritation.

4.1.10.2 Humans (Summary)

Reproductive and developmental toxicity of formaldehyde in humans has also been reviewed by Nielsen *et al.* (2013). The authors discussed two putatively contradicting reviews and meta-analyses published by Collins *et al.* (2001) and Duong *et al.* (2011) and came to the conclusion that the results from both studies were not that substantially different. In their overall evaluation they concluded that there was no indication for reproductive or developmental toxicity of formaldehyde in pregnant women. The authors also discussed the limited data available on paternal toxicity and concluded that there was also no convincing evidence in the literature that paternal fertility or reproduction (transmissed via female mates) would be anyhow being affected by formaldehyde via inhalative exposure. This result is in agreement and supported by toxicokinetic studies indicating that formaldehyde does not reach the internal organs (see section 3.3.9).

4.1.11 Toxicokinetics

4.1.11.1 Endogenous occurrence

Formaldehyde is present at low levels in most living organisms. It is an endogenous metabolite with measurable levels in body fluids and tissues in mammalian systems. Physiological amounts of formaldehyde are formed from serine, glycine, methionine and choline by demethylation of N-, O-, and S-methyl compounds (IARC, 1995; IPCS, 2002). It is an essential intermediate in the biosynthesis of purines, thymidine and certain amino acids (IARC, 1995). Although formaldehyde is a gas at room temperature, it hydrates rapidly and is in equilibrium with its hydrated form methanediol. Formaldehyde is rapidly metabolised to formic acid (formate) mainly subsequently to formation of a glutathione conjugate. Formate is metabolised and either incorporated via normal metabolic pathways into the one-carbon (C1) pool or further oxidised to CO_2 and exhaled.
The mean endogenous concentration of free and reversible bound formaldehyde in blood of unexposed humans was 2.61 µg/g blood (range 2.05 – 3.09 µg/g = mg/l), in rats 2.24 µg/g and in monkeys 2.42 µg/g (Casanova *et al.*, 1988; Heck *et al.*, 1985), i.e., about 0.1 mM.
In livers or nasal mucosa of rats formaldehyde concentrations of 200 – 400 µmol/l were determined (Heck *et al.*, 1985). Very recently, a value of 0.1 mM (3 ppm) was analysed in the blood of unexposed humans, monkeys and rats within the frame of developing of a non-destructive analytical method to determine the free formaldehyde levels (Tallon *et al.*, 2009). This is in line with data published by Cascieri and Clary (1992) who stated that the average blood level of formaldehyde in both exposed and unexposed animals and humans is about 2.5 ppm.

The widespread distribution in the body was supported by measured formaldehyde levels of 1.5 – 15 mg/kg in various tissues. It is also estimated that the level of free formaldehyde is 1 – 2% of the total formaldehyde level (Cascieri and Clary, 1992). The half-life and the wide distribution of formaldehyde in the body and blood enable an estimate of the body's normal daily formaldehyde production, turnover or use. Assuming an equilibrium level in aqueous systems of the body of 2.5 ppm (2.5 mg/l), the body level of total formaldehyde at any one time is approximately 122.5 mg (2.5 mg/l x 49 l) with an average total formaldehyde tissue level of 1.75 mg/kg bw, which is in the same range as reported by Heck, 1982 (in Cascieri and Clary, 1992). The half-life of 1.5 min means that half of the 122.5 mg (61.25 mg) will be used up in 1.5 min (transferred to C1 pool or excreted as CO_2) and that an equal amount of formaldehyde will be produced by the body to maintain the 2.5 ppm blood level. This suggests that the human body produces 2,450 mg formaldehyde per h (61.25 mg x 60 min divided by 1.5 min) or 58,000 mg/day. Owen *et al.* (1990) calculated that an adult human liver will convert 22 mg formaldehyde per min directly to CO_2, that is 1,320 mg per h.

4.1.11.2 Absorption

4.1.11.2.1 Oral route

There is an old study with limited validity available on the toxicokinetics in rats and mice after oral administration (Buss *et al.*, 1964). Rats and mice were gavaged with [^{14}C]-formaldehyde (no further details reported) and radioactivity was determined in expired air, urine and feces (no further details reported). Formaldehyde was rapidly and nearly completely absorbed from the intestinal tract after gavage. Approximately 50% of the dose were metabolized and excreted as CO_2 via exhaled air. Within 12 h 10% of the radioactivity was excreted via the urine and 1% via faeces.

4.1.11.2.2 Inhalation route

1st study: Heck *et al.*, 1985

Guideline:	No
Species/strain:	Rat (Fischer 344), Human Volunteers
Group size:	8 per group (rats), 6 human volunteers (4 males, 2 females)
Test substance:	[$^{13}C^{2}H_2$]-Formaldehyde
Batch:	no data
Purity:	no data
Route:	Inhalation
Dose level:	14.4 ± 2.4 ppm (rats), 1.90 ± 0.06 ppm (humans)
Exposure period:	2 h (rats), 40 min (humans)
Exposure conditions:	Rats: nose only (no data on generation of specific concentration or analytical control). Humans: volunteers were exposed in walk-in-chambers; temperature: 23°C, RA humidity 50% (no further data on generation of specific concentration or analytical control).
Sampling:	Rats: immediately after exposure and decapitation (2 x 1.5 ml blood). Humans: venous blood (3 x 1.5 ml) prior to and after exposure
Determination:	Addition of acidic pentafluorophenylhydrazine (PFPH) and a known amount of [$^{13}C^{2}H_2$]-formaldehyde (as internal standard) in NaOH solution (pH 11). [$^{13}C^{2}H_2$]-formaldehyde concentration determined by chromotropic acid method; suspensions incubated at 50°C for 2 h to form the corresponding pentafluorophenylhydrazones, which were extracted; extracts analysed by GC/MS using selected ion monitoring; the ions correspond to the PFPH derivatives of formaldehyde and [$^{13}C^{2}H_2$]-formaldehyde. Free and reversibly bound formaldehyde measured.

GLP: no

Results and discussion:
In rats exposed to 14.4 ppm (17.3 mg/m^3) formaldehyde for 2 hours, a blood concentration of 2.25 ± 0.07 µg/g was measured immediately after the end of exposure vs. 2.24 ± 0.07 µg/g in controls. Similarly, no difference was found between blood concentrations of formaldehyde before exposure (2.61 ± 0.14 µg/g) and immediately after exposure (2.77 ± 0.28 µg/g) of human volunteers against 1.9 ppm (2.3 mg/m^3). However, the volunteers differed with respect to their blood formaldehyde concentration (for some individuals, blood levels of formaldehyde raised after exposure while it decreased in others) suggesting individual variations. The lack of the increase in the endogenous formaldehyde concentration can be considered as indication for rapid metabolism of the inhaled portion. Absence of an increase in blood concentration further to inhalation might be due to its deposition within the respiratory tract and its rapid metabolism in the nasal mucosa. In animal species, the half-life of formaldehyde administered intravenously ranges from approximately 1 to 1.5 min in the circulation.

2nd study: Casanova *et al.*, 1985

Guideline:	No
Species/strain:	Rhesus monkey (*Macaca mulatta*)
Group size:	3 per group
Test substance:	[$^{13}C^2H_2$]-Formaldehyde
Batch:	no data
Purity:	no data
Route:	Inhalation
Dose level:	6.00 ± 0.22 ppm
Exposure period:	6 h/day, 5 days per week for 4 weeks
Exposure conditions:	15 m^3 chamber. Gas generated via thermal depolymerisation of paraformaldehyde; concentration monitoring by infrared spectrophotometer
Sampling:	Immediately (7 min) after last exposure (3 ml blood) and after 45 h of recovery.
Determination:	Addition of acidic pentafluorophenylhydrazine (PFPH) and a known amount of [$^{13}C^2H_2$]-formaldehyde (as internal standard) in NaOH solution (pH 11). [$^{13}C^2H_2$]-formaldehyde concentration determined by chromotropic acid method; suspensions incubated at 50°C for 2 h to form the corresponding pentafluorophenylhydrazones, which were extracted; extracts analysed by GC/MS using selected ion monitoring; the ions correspond to the PFPH derivatives of formaldehyde and [$^{13}C^2H_2$]-formaldehyde. Free and reversibly bound formaldehyde measured.
GLP:	no

Results:
No statistical difference between the two measures: 1.84 ± 0.15 µg/g after 7 min and 2.04 ± 0.40 in µg/g after 45 h (p=0.33).

3rd study: Kleinnijenhuis *et al.*, 2013

Guideline:	According to OECD TG 403
Species/strain:	Rat (Sprague-Dawley)
Group size:	10 males per group
Test substance:	[^{13}C]-formaldehyde
Batch:	cx1586 (19.3% in aqueous solution, 99.4 atom % [^{13}C])
Purity:	no data
Route:	Inhalation
Dose level:	10 ppm

Exposure period:	6 h and 3 min (3 min for blood collection)
Exposure conditions:	Nose-only exposure chamber, atmosphere was analytically monitored (mean concentration during exposure: 9.65 ± 0.44 ppm)
Sampling:	Prior to exposure, after 3 h exposure, at the end of exposure and 10, 30 min afterwards
Determination:	LC-MS/MS after derivatisation with 2,4-dinitrophenylhydrazine to give the formaldehyde-DNPH adduct.
GLP:	yes

Results and discussion:
The inhalation of [^{13}C]-formaldehyde at 10 ppm had no significant effect on the total formaldehyde concentration in blood. Exogenous [^{13}C]-formaldehyde was not detected in the blood stream of exposed rats during or after inhalation exposure for 6 h and 3 min under the experimental conditions of this study. The applied method would have allowed the detection of exogenous [^{13}C]-formaldehyde in blood at a concentration approximately 1.5% of the endogenous formaldehyde blood concentration.

Overall conclusion on inhalative absorption (according to IARC, 2006, 2012):

Consistent with its high water solubility and reactivity with macromolecules, formaldehyde is deposited and absorbed after inhalation in the upper respiratory tract (site of 1st contact). The amount was found directly proportional to the concentration. Differences between species were found in the actual sites of uptake; in obligate nose breathers like rats absorption occur in the nasal passages and in oronasal breathers like humans and monkeys in nasal passages but also in oral passages, trachea, and proximal bronchi (IARC, 1996, IPCS, 2002). The overall uptake by the nasal passages has been predicted to be 90% in rats, 67% in monkeys, and 76% in humans (Schulte *et al.*, 2006). Further, >93% of a dose (2, 6, 15, or 50 ppm) of inhaled radiolabelled formaldehyde was absorbed by the tissues of the rat nasal cavity, regardless of the airborne concentration (Schulte *et al.*, 2006)
Generally, the localisation of uptake in each species is determined by nasal anatomy, mucus coating and clearance mechanisms. It could be demonstrated for the rat model that the main part of flow intake at the nostrils passes into the middle and lateral meatuses with less flow to the dorsal and ventro-medial pathways (Schulte *et al.*, 2006). Results that have been confirmed by data demonstrating histopathological damage and DPX formation at these sites. Similar results were presented for the monkey. So, it was shown that formaldehyde following inhalation is absorbed and deposited in the upper respiratory tract, the site of first contact, but the physiological level of formaldehyde in the blood of humans and experimental animals is not increased due to its rapid oxidation to formic acid and reactivity at the site of first entry.
A mathematical model for the absorption and metabolism of formaldehyde in humans (Franks, 2005) suggests that at inhaled concentrations of 1.9 ppm, the flux of formaldehyde to the blood increases rapidly at the beginning of exposure, reaching a constant magnitude within a few seconds. The predicted amount of inhaled formaldehyde entering the blood is very small (maximum of 15 µg/l during repeated daily 8-hrs exposures). These results are consistent with the absence of variation of blood endogenous concentrations being around 2.61 ± 0.14 mg/l (2,000 – 3,000 µg/l) further to exposure to 1.9 ppm for 40 min in 6 volunteers (Heck *et al.*, 1985). The predicted increase represents only 0.016% of this pre-exposure value. The simulation of exposure to 1.9 ppm for 8 h/day, 5 days/week predicted a constant maximum concentration in the blood at the same level, with a quick removal from the blood after exposure.

4.1.11.3 Distribution, metabolism and excretion

4.1.11.3.1 Distribution – Dermal exposure

Study: Jeffcoat *et al.*, 1983

Guideline:	According to OECD TG 417
Species/strain:	Rat (Fischer 344), guinea pig (Dunkin-Hartley), monkey (*Cynomolgus*)
Group size:	3 per group
Test substance:	[^{14}C]-formaldehyde dilution (in 37% formaldehyde)
Batch:	no data
Purity:	no data
Route:	Dermal
Exposure:	One day prior to the experiment rats and guinea pigs received a catheter implanted in the carotid artery and skin was shaved. 10 µL containing 0.1 mg of ^{14}C-formaldehyde or 40 µL containing 11.2 mg of ^{14}C-formaldehyde were applied to a 2 cm² area of the lower back (presumably non-occlusive); blood samples collected 1, 2, 3, 4, 7, 24 h after dosing. 72 h after application animals were sacrificed. Similar methods used for monkeys but placed in a restraining chair with plexiglas hood. 200 µL containing 2 mg test substance (590-730 µCi) applied to 18 cm² shaved area
Sampling & method:	Urine and feces collected at daily intervals for 3 days. Animals were sacrificed 72 hours after dosing, and tissue samples from the heart, liver, lung, spleen, kidney, leg, brain, gonads, skin at the application site, distant skin, and the remaining carcass were analysed for ^{14}C content by scintillation counting.
GLP:	no

Results:
There was no accumulation of ^{14}C in any tissue in any species. Blood concentrations were stable throughout the experiment, averaging at about 0.015% of the administered dose in monkeys and at about 0.1% of the dose in rats and guinea pigs. In rats and guinea pigs, about 4.5% to 8.3% of the applied radioactivity was detected in the urine, 0.7% to 1.5% in the faeces, and 21.4% to 28.3% in the air traps; 22.2% to 28.4% remained in the carcass.
The amount of radioactivity remaining in the skin ranged from 3.8 to 15.6% in guinea pigs and 3.4 to 16.2% in rats.
In monkeys, about 0.24% of the applied dose was excreted in the urine, 0.2% was excreted in the faeces, 0.37% was exhaled, and about 9.5% remained in the skin at the site of application. No data for the remaining in the carcass of monkeys were provided. The authors concluded that the skin of monkeys was less permeable to formaldehyde than that of rodents, and that the large majority of applied radiolabel was lost due to evaporation.

4.1.11.3.2 Distribution – Inhalation exposure

1st study: Chang *et al.*, 1983

Guideline:	no
Species/strain:	Rat (Fischer 344), mouse (B6C3F1)
Group size:	3 males per group
Test substance:	[^{14}C]-formaldehyde along with unlabelled paraformaldehyde
Batch:	no data
Purity:	no data
Route:	Inhalation

Concentration:	15 ppm
Exposure:	Whole body exposure. Gas generated by thermal depolymerisation of paraformaldehyde in a beaker and mixed with air before introduction into the chamber. Concentration monitored by infrared spectrophotometer.
Sampling & method:	Single exposure of 6 h with the labelled substance (pre-exposure: 15 ppm, 6 h/day for 4 days with unlabelled test substance) in a whole body exposure chamber. Animals were killed immediately after inhalation exposure and heads prepared for cross sectioning (3 animals per species and pretreatment); further animals used for longitudinal sectioning of the whole body autoradiography (3 animals per species and pretreatment). 50 µm thick cryo-sections used for autoradiography.
GLP:	no

Results:
Comparable amounts of radioactivity were detected in naive and pretreated (15 ppm, 6 h/day for 4 days) rats, but less radioactivity in visceral tissues in pre-treated rats. Although the rats were sacrificed immediately after inhalation period, widespread distribution of radioactivity was visible in autoradiographs of longitudinal sections. Higher amounts were observed in nasal cavity, trachea, lung and gastro-intestinal tract. In naive mice without pre-treatment more radioactivity was deposited in the nasal cavity and less radioactivity in visceral tissue in comparison to pre-treated mice (15 ppm, 6 h/day for 4 days). Although mice were sacrificed immediately after inhalation period, widespread distribution of radioactivity was visible in autoradiographs of longitudinal sections. Higher amounts were observed in nasal cavity, trachea, lung and gastro-intestinal tract.

Conclusions:
Beside the high amounts of radioactivity in nasal cavity, trachea, lung and gastro-intestinal tract a widespread distribution of radioactivity was seen in mice and rats exposed for 6 h to 15 ppm [^{14}C]-formaldehyde and sacrificed immediately after the exposure period. Formaldehyde and/or metabolites are widely distributed in the body of rats and mice after inhalation exposure.

2nd study: Heck *et al.*, 1983

Guideline:	OECD TG 417
Species/strain:	Rat (Fischer 344)
Group size:	≥4 males per group
Test substance:	[^{14}C]-formaldehyde along with unlabelled paraformaldehyde
Batch:	no data
Purity:	no data
Route:	Inhalation
Concentration:	5-24 ppm
Exposure:	Dynamic conditions; head only exposure. Gas was generated by thermal depolymerisation of paraformaldehyde in a flask and mixed with air before introduction into the chamber. Concentration monitored by infrared spectrophotometer or by chromotropic acid method. Specific activities measured by scintillation counting. Single exposure of 6 h with the labelled substance (pre-exposure: n=4, 6 h/day for 9 days with unlabelled test substance (15 ppm) in a whole body exposure chamber).
Sampling & methods:	Distribution: 4 rats per dose (2 replicates; total 12 rats per dose) were exposed for 6 h to 5, 10, 15, 24 ppm [^{14}C]-formaldehyde. Immediately after exposure the rats were sacrificed and tissue

samples collected. Tissues were homogenized and prepared for scintillation counting for analysis of radioactivity.
Metabolism: 4 rats per dose were exposed for 6 h to 0.63 or 13.1 ppm [^{14}C]-formaldehyde. Immediately after exposure the rats were transferred to metabolism cages (collection of expired air, urine, faeces) for 70 h. CO_2 was trapped for determination of $^{14}CO_2$ excretion. ^{14}C in urine and faeces determined. Carcass was measured for residual radioactivity as well as cage washings.
Toxicokinetics (blood): Uptake and disappearance of radioactivity in blood was measured during and after inhalation exposure (8 ppm, 6 h, [^{14}C]-formaldehyde, n=1). In further experiments rats received i.v. 25 µCi [^{14}C]-formaldehyde or 29 µCi [^{14}C]-formate.

GLP: no

Results:

Disposition of radioactivity in various fractions after 6 h inhalation exposure to 0.63 or 13.1 ppm 14C-formaldehyde (n=4 per group, rats sacrificed 70 h after exposure; mean±SD)

	Percentage of radioactivity	
	0.63 ppm	**13.1 ppm**
Expired air	39.4±1.5	41.9±0.8
Urine	17.6±1.2	17.3±0.6
Feces	4.2±1.5	5.3±1.3
Tissues (including nasal mucosa) and carcass	38.9±1.2	35.2±0.5

The amount of radioactivity was highest in the nasal mucosa of rats compared to other tissues indicating absorption primarily via the upper respiratory tract. Pre-exposure of rats did not alter the absorption in nasal mucosa or plasma (low values in plasma: approximately 80 nmoles/g vs. ~2,200 nmoles/g in nasal mucosa). The concentration of radioactivity in other organs was similar to that in plasma and also low in comparison to the nasal mucosa.
After inhalation of radioactive formaldehyde in the rat, radioactivity is mainly exhaled as CO_2 during the 70-h post-exposure period (40%) and excreted in the urine (17%). 35 – 39% remained in the tissues (carcasses) presumably as products of metabolic incorporation in macromolecules (C1 pathway). The exposure concentration had no influence on the relative amount in the different fractions.

3rd study: Casanova *et al.*, 1989

Guideline: no
Species/strain: Rat (Fischer 344)
Group size: 4 males per group
Test substance: [^{14}C]-paraformaldehyde and [^{3}H]-formaldehyde (13.5-72 mCi/mM)
Batch: no data
Purity: no data
Route: Inhalation
Concentration: 6 ppm
Exposure period: 6 h
Exposure: Nose only, acclimatisation of rats 1 h before exposure; vaporizing of ^{14}C-paraformaldehyde or ^{3}H-formaldehyde together with unlabelled test substance in a 50 l teflon bag; analytical control of formaldehyde concentration (IR spectrophotometry) and specific ^{14}C and ^{3}H activities (scintillation counting).

Sampling & method: Nasal mucosa, respiratory epithelium (tissues from 4 rats combined). Determination of labelled DNA: HPLC and scintillation counting.
GLP: no

Results:
HPLC analysis of the DNA from the nasal mucosa of rats exposed for 6 h to 6 ppm radiolabelled formaldehyde revealed a high amount of [^{14}C] in the respiratory mucosa due to metabolic incorporation. Approximately 91% of the [^{14}C] was found in the DNA (32% in dG, 40% in dT, 19% in dA, not detected in dC). The remaining 9% contributed to DPX.

4th study: Casanova-Schmitz *et al.*, 1984, Casanova and Heck 1987
Guideline: no
Species/strain: Rat (Fischer 344)
Group size: 8 males per group
Test substance: Formaldehyde
Batch: no data
Purity: no data
Route: Inhalation
Concentration: 15 ppm
Exposure period: 6 h per day for 10 days
Exposure: 8 m³ chamber, airflow 1.7 m³/min, 74±1°F, 45% rel. air humidity. Gas was generated by thermal depolymerisation of paraform-aldehyde in an isothermal oven and mixed with air before introduction into the chamber. Concentration monitored by infrared spectrophotometer.
Sampling & method: Nasal mucosa (respiratory and olfactory epithelium, tissues of 8 rats combined). Rate of oxidation of formaldehyde in the presence and the absence of glutathione (GSH) was determined. The free GSH concentration was kept constant (1.5 mM). Estimation of Michaelis-Menton constants V_{max} and K_m.
GLP: no

Results:
Under the conditions of the study, formaldehyde was oxidized by nasal mucosal homogenates and formaldehyde dehydrogenase and acetaldehyde dehydrogenase as identified in homogenates of respiratory and olfactory tissues from rat nasal cavity. Repeated exposure of rats did not change the specific activity of the dehydrogenases. GSH is a cofactor for formaldehyde dehydrogenase. GSH and formaldehyde react reversibly and form S-hydroxymethyl-GSH and this adduct is the acutal substrate for formaldehyde dehydrogenase. Formaldehyde dehydrogenase (K_M = 2 – 4 µM) in the presence of GSH is more effective in oxidation of formaldehyde than aldehyde dehydrogenase (K_M = 450 – 650 µM). The specific activity of formaldehyde dehydrogenase in the olfactory mucosa was about twice that in the respiratory mucosa; specific activity of aldehyde dehydrogenase was similar in both tissues. GSH-dependent oxidation of formaldehyde catalyzed by formaldehyde dehydrogenase is an important defense mechanism against the formation of covalent binding of formaldehyde to DNA.

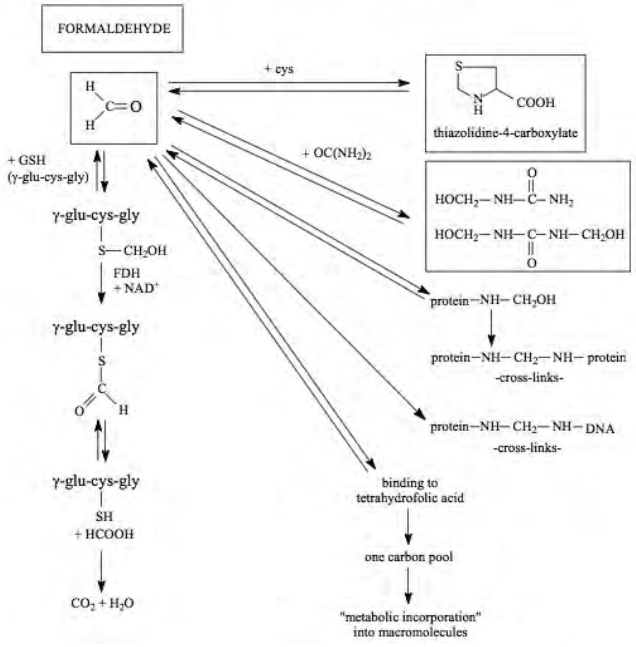
4.1.11.3.3 Metabolism

As reviewed by Schulte *et al.* (2006) enzymatic oxidation of formaldehyde results in detoxification from elevated endogenous and exogenous formaldehyde concentrations. Systems involved: Spontaneous and non-enzymatic reaction with GSH to produce S-hydroxymethyl-GSH. S-hydroxymethyl-GSH is oxidized by cytosolic formaldehyde dehydrogenase to form S-formyl-GSH (Uotila *et al.*, 1997). The cytosolic formaldehyde

dehydrogenase is present in all animal tissues and in human liver, brain, oral mucosa, or erythrocytes. Formaldehyde dehydrogenase was also detected in the respiratory and the olfactory epithelium of the nasal cavity in rats. However, the literature data are insufficient for explaining the differences in the affected regions in the nose (lesions predominantly in the respiratory epithelium and less in the olfactory epithelium of the nasal cavity). Unfortunately, it has not yet been investigated whether and to which extent formaldehyde dehydrogenase is active in human nasal and/or pharyngeal tissue.
In the absence of GSH formaldehyde oxidation in tissues of rats was catalysed by an isoenzyme of aldehyde dehydrogenase. Oxidation by catalase is a further possible pathway important after depletion of GSH. However, in the presence of GSH, formaldehyde dehydrogenase is more effective in oxidising formaldehyde than aldehyde dehydrogenase.
Data on repeated dose toxicity have shown a sharp increase in toxicity. Lesions of the epithelium in the nasal cavity can be observed at formaldehyde concentration ≥6 ppm. This is in accordance with pharmacokinetic data (Casanova *et al.*, 1989): The detoxification pathway (mainly via formaldehyde dehydrogenase) in rats is half saturated at an airborne formaldehyde concentration of 2.6 ppm (Casanova *et al.*, 1989). Formaldehyde dehydrogenase catalyses the formation of S-formyl-GSH (cf. above). S-formyl-GSH is then enzymatically hydrolysed to formic acid and GSH (Uotila *et al.*, 1997). Enzymes involved are S-formyl-GSH hydrolase and glyoxalase II, both of which with ubiquitous tissue distribution.
Formic acid can be excreted via urine as its sodium salt, or cleaved to H_2O and CO_2, which is exhaled. As formate, an uptake into the C1 metabolic pathway is possible.

Further biological pathways taken from Schulte *et al.*, 2006 and IARC, 2006:

Figure 1. Biological reactions and metabolism of formaldehyde

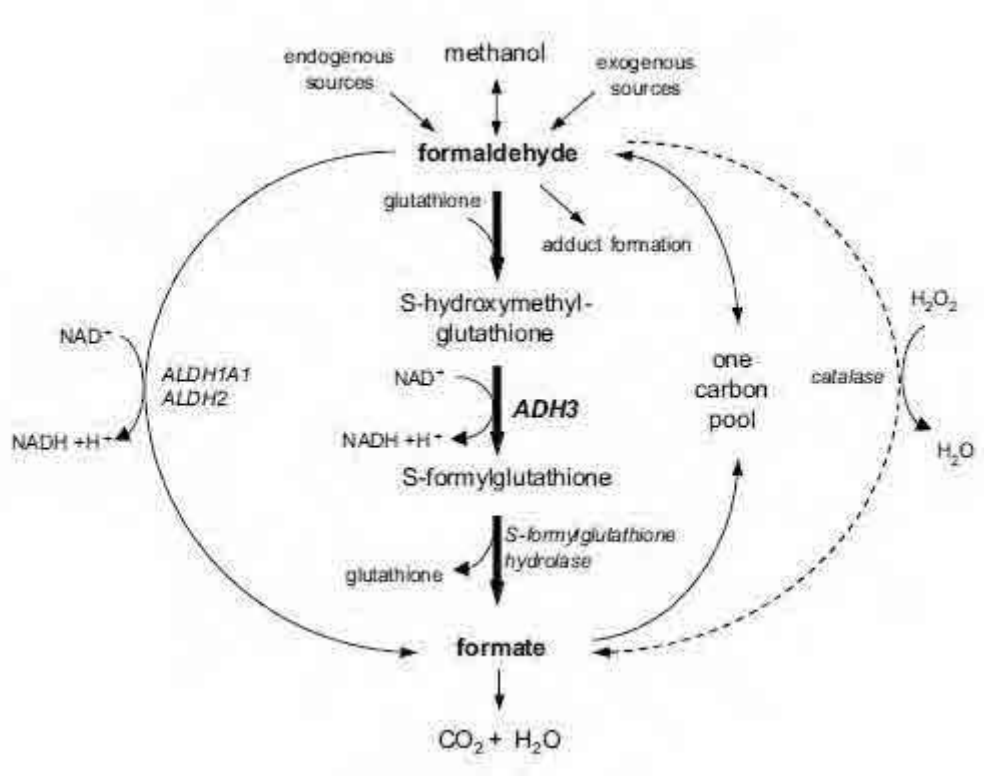


1) Reversible binding of formaldehyde to cysteine resulting in the formation of thiazolidine 4-carboxylate.
2) Reversible binding to urea to form hydroxymethyl adducts.
3) Reversible binding mucus proteins in the nasal or oral mucosa.
4) Irreversible reaction with two proteins resulting in protein-protein cross-links.
5) Irreversible reaction with one protein and DNA resulting in DPX.
6) Non-enzymatic binding to tetrahydrofolic acid followed by an uptake into the C1 metabolic pathway and incorporation into biological macromolecules (synthesis of purine, thymidine and certain amino acids).

4.1.11.3.4 Excretion

Rats were exposed for 6 h to 0.63 or 13.1 ppm [^{14}C]-formaldehyde (Heck *et al.*, 1983, see 3.3.9.3.2). Immediately after exposure the rats were transferred into metabolism cages for 70 hours. Most of radioactivity was excreted via exhalation (~40%). Nearly the same amount remained in the tissues and carcasses. 17% were excreted via urine and 5% via faeces. The exposure concentration had no influence on the relative amount in the different fractions. The excretion via expired air was multiphasic with an initial high rate of exhalation, which declined rapidly over a period of 12 h and followed by a much slower phase.

Summary Toxicokinetics (according to IARC, 2006, 2012):



Formaldehyde is an intermediate in the C1 pool and is present in measurable concentrations in all metabolically active cells and tissues (Heck *et al.*, 1982, 1985; Casanova *et al.*, 1988). In aqueous solution, formaldehyde is rapidly converted to its diol form, methanediol (formaldehyde hydrate, $CH_2(OH)_2$, methylene glycol), which enters in a dynamic equilibrium with formaldehyde. The concentrations of the diol and of formaldehyde depend on the conditions (temperature, pH, formaldehyde concentration) under which the reaction occurs. Importantly, methanediol, with a molecular weight of only 48, can readily penetrate into tissue (Fox *et al.*, 1985).

The absorption of formaldehyde occurs readily in the upper respiratory tract (Casanova *et al.*, 1991; Kimbell *et al.*, 2001a,b). Once inhaled, formaldehyde can react directly with mucus or with macromolecular cellular components including proteins and nucleic acids; it can be incorporated into biological molecules through folate-dependent enzymatic processes; it can be oxidized to formic acid or to CO_2 through enzymatic processes dependent on formaldehyde dehydrogenase, aldehyde dehydrogenase and, in limited situations, catalase (Hedberg *et al.*, 2002), or it can be exhaled. It has been estimated that as much as 22–42% of inhaled formaldehyde may be removed by mucus flow (Schlosser, 1999).

Formaldehyde reacts readily and reversibly with amino groups to form Schiff bases, and with sulfhydryl groups resulting in the formation of S-hydroxymethyl-GSH, which is oxidized by formaldehyde dehydrogenase (that is, alcohol dehydrogenase-3, ADH3) to S-formyl-GSH. The latter is further metabolized by S-formyl-GSH hydrolase to generate formate and gluthione. The formate can also be formed non-enzymatically (Hedberg *et al.*, 2002).
Incubation of 0.1 – 5 mM formaldehyde with reduced GSH in solution followed by addition to dG or to calf thymus DNA leads to the formation of the relatively stable adduct S-[1-(N^2-dG)-methyl]-GSH (Lu *et al.*, 2009). This adduct may form endogenously, as both formaldehyde and reduced GSH are present in reasonably high concentrations within cells. It may also serve as a biomarker to study the penetration of inhaled formaldehyde and to distinguish endogenous from exogenous formaldehyde-derived adducts.
No change in formic acid concentration was observed in the urine of medical students over a 3-wk period during which they were exposed to air concentrations <0.5 ppm (0.62 mg/m^3) (Gottschling *et al.*, 1984). Similarly, no statistically significant change in the concentration of formaldehyde in blood was found after inhalation of this substance at 1.9 ppm (2.34 mg/m^3) for 40 min by six human volunteers; at 14.4 ppm (17.8 mg/m^3) for 2 h in rats (Heck *et al.*, 1985); and at 6 ppm (7.4 mg/m^3) for 6 h/day, 5 days/wk, for four weeks in Rhesus monkeys (Casanova *et al.*, 1988). Blood was drawn approximately 7 min and 45 h after the end of the exposure period, from monkeys whose blood levels were 1.84 ± 0.15 µg/g and 2.04 ± 0.40 µg/g, respectively.
Studies on the uptake of radiolabelled formaldehyde by inhalation, ingestion and through the skin do not provide information that would help to determine whether unreacted formaldehyde reaches the bone marrow, because it is rapidly taken up in the C1 pool and incorporated into macromolecules.

4.1.12 Human data

4.1.12.1 Acute toxicity

Pandey *et al.* (2000) reviewed on the toxicity of ingested formalin in accidental, homicidal or suicidal attempts. Ingestion of formaldehyde may cause burning in the mouth and oesophagus, nausea and vomiting of tissue and blood or coffee ground material, abdominal pain, and diarrhea. Further it can cause liver and kidney damage, leading to jaundice albuminuria, haematuria and anuria, acidosis and convulsions or central nervous system depression. Ultimately it can lead to unconsciousness and death resulting from cardiovascular failure. The fatal dose in humans is about 60 – 90 ml.
According to ATSDR (1999), a woman ingested formaldehyde in a suicide attempt and was presenting in coma. Lethargy, seizure and loss of consciousness was observed in a case after ingestion of 517 mg/kg bw, and loss of consciousness in a woman after 624 mg/kg bw.

4.1.12.2 Irritation

4.1.12.2.1 Skin irritation

It has been estimated that a single occlusive application of 1% formaldehyde in water will produce an irritant response in approximately 5% of the human population (WHO, 1989). In ATDSR (1999), no irritant effects were observed in humans at a concentration of 1%. Thus, irritant effects were expected at concentrations >3%.

4.1.12.2.2 Eye and respiratory irritation

Exposure to gaseous formaldehyde results in irritation of eyes, nose and throat (DFG, 2000; IPCS, 2002). There is some evidence that eye irritation is the most sensitive endpoint. Slight discomfort due to irritation (mainly eyes) was noted in some individuals at a concentration as low as 0.3 mg/m³ (0.25 ppm) (Andersen and Molhave, 1983; Holmström *et al.*, 1989); other data suggest that in 10 volunteers no eye irritation occurred at 0.5 ppm but odour was detected; dose-dependent eye irritation was reported at ≥1 ppm for 3 h (Kulle *et al.*, 1987, Kulle, 1993).

Besides subjective rating of symptoms as investigated in the studies mentioned, also objective ratings for eye irritation (conjunctival redness and eye blinking frequency) have been investigated. Healthy volunteers were exposed to 0, 0.15, 0.3 ppm, 0.3 ppm plus 4 peaks of 0.6 ppm, 0.5 ppm, or 0.5 ppm plus 4 peaks to 1.0 ppm (Lang *et al.*, 2008). In this study the NOAEL was 0.5 ppm for the objective measures (the LOAEL was reached at additional peaks of 1 ppm) while very slight subjective symptoms (probably influenced by the perception of the odour of formaldehyde) were reported already at 0.3 ppm. No subjective symptoms were noted at 0.15 ppm. The authors stated that "the subjective complaints of ocular and nasal irritation noted at lower levels were not paralleled by objective measurements of eye and nasal irritation and were strongly influenced by personality factors and smell". They concluded that the NOEL for subjective and objective eye irritation was 0.5 ppm in case of a constant exposure level. The overall NOAEL in this study is 0.5 ppm without exposure peaks and 0.3 ppm with exposure peaks of 0.6 ppm.

The odour threshold in most humans is below 1 ppm. However, the individual detection threshold covers a wide range in several studies available on this endpoint. In a group of 50 subjects the 50-percentile detection threshold was 0.145 ppm, the 10-percentile detection threshold 0.020 ppm and the 90-percentile threshold 0.5 ppm (IARC, 1995; WHO, 1989). In studies on volunteers subjective symptoms are evaluated and no objective differentiation between odour perception and irritation of eyes, nose and throat is given. Highest data quality is reported in chamber studies under controlled conditions (DFG, 2000). As stated above eye irritation seems the most sensitive endpoint which occurs in some people even at a concentration of 0.25 ppm (Andersen and Molhave, 1983; Paustenbach *et al.*, 1997; Arts *et al.*, 2006). Others reported values of ≥1 ppm (Kulle *et al.,* 1987). Summarising these data, the German MAK commission concluded in 2000: Significant increases in eye irritation effects are expected at ≥1 ppm (DFG, 2000); however, slight eye irritation might occur in susceptible individuals already at 0.25 ppm. Long-term exposure of subjects prior to the test did not alter the results in comparison to unexposed subjects and there was no difference in sensitivity between asthmatics and healthy volunteers (DFG, 2000). By contrast, nose and throat irritation is thought starting to occur at dose levels of ≥2 ppm (DFG, 2000; IPCS, 2002).

4.1.12.2.3 Special effect: Lung function parameters after inhalation exposure

In studies on workplace exposures respiratory parameters such as FEV_1 (forced expiratory volume in one second) or FVC (forced vital capacity) were also found changed. Such effects were reported at average concentrations of 1 – 2 ppm formaldehyde (DFG, 2000). However, in controlled chamber studies on volunteers (no workplace) no such effects on lung function were detected at concentrations up to 3 ppm (Andersen and Molhave, 1983, Kulle, 1993). Further, in asthmatic people no increase in pulmonary dysfunction was evident in controlled studies at concentrations up to 3 ppm (Schulte *et al.*, 2006).

It has been concluded that lung function is affected at workplaces with formaldehyde concentrations higher than 1 ppm, whereas no such effects have been reported in controlled human exposure studies at concentrations up to 3 ppm.

4.1.12.3 Sensitisation

4.1.12.3.1 Skin

Formaldehyde is a primary skin sensitizer inducing allergic contact dermatitis Type IV and may induce contact urticaria Type I (WHO, 1989). However, contact urticaria has been rather rarely associated with formaldehyde exposure (IARC, 1995).
Concentrations of 1-2% (Ponten et al., 2013) or less elicited positive reaction in approximately 2% of all patients tested throughout the world in dermatology clinics; higher concentrations used for challenge might be irritant (WHO 1989; ATSDR 1999). Formaldehyde-induced contact dermatitis is concentration- and patch test condition-dependent. In human repeat insult patch tests (HRIPT) different induction concentrations of formaldehyde ranging from 0.1% to 10% were assessed (Marzulli and Maibach, 1974). Positive responses started at 1% (4.5% of the patients positive). At a concentration of 10% 7.8% of the subjects showed positive responses. A threshold concentration for induction has been estimated to be less than 5% aqueous solution (OECD, 2002). In this OECD review, a threshold for the challenge concentration in patch tests on formaldehyde sensitized subjects was reported at 30 ppm (0.003%) in aqueous solution and 60 ppm (0.006%) for products containing formaldehyde. However, others suggested positive reactions to formaldehyde to be rare below concentrations of 0.025 – 0.05% (ATSDR, 1999).
A 10 year multicentre analysis of the European Environmental and Contact Dermatitis Research Group (EECDRC) on the frequency in patch-tested patients of contact allergy to common preservatives collected in 16 centers in 11 countries during the years 1991 – 2000 showed stable levels (~2-3 %) of sensitivity to formaldehyde (Wilkinson *et al.*, 2002). The subsequent follow-up analysis during the years 2001 – 2008 in 12 European centers showed a prevalence of allergy to formaldehyde of 1.5 – 2.6% (Svedman *et al.*, 2012). Similar numbers are published as results of a European Surveillance System on Contact Allergies (ESSCA) from 2005/2006 (Uter *et al.*, 2009) and in an update covering the period 2007 – 2008 (Uter *et al.*, 2012). This ESSCA study, which covered 39 departments in 11 European countries demonstrated frequencies of 0.7 – 3.6% for formaldehyde in the different countries.
The sensitisation rate in European patients seems to remain stable over the last several years as demonstrated by the German IVDK (Schnuch *et al.*, 2008, 2011). A ten year prevalence was extrapolated: More than 200,000 patients were patch-tested and the sensitisation prevalence to formaldehyde was 1.54% in this clinical population. In the general population, however, the prevalence of contact allergic reactions to formaldehyde is estimated to be below 0.5% (personal communication).

4.1.12.3.2 Repiratory tract

According to ATSDR (1999), a few case reports of bronchial asthma were reported (2 renal dialysis nurses, a plastic molder, a printer, a worker in a phenol formaldehyde manufacturing plant, and a carpenter) that point to respiratory tract sensitisation. In acute exposure challenges at exposure levels <3 ppm all above mentioned subjects showed marked changes in FEV_1 or airflow rates. However, in a study on 230 patients occupationally exposed to formaldehyde and who had reported respiratory symptoms consistent with asthma, only 12 subjects showed a decrease of >15% in the peak expiratory flow rate (PEFR) after acute challenge with 2 ppm formaldehyde (ATSDR, 1999; WHO, 1989). By contrast, no challenge-induced deficits in FEV_1 or airflow rates were demonstrated in 3 other studies on formaldehyde-exposed subjects with respiratory problems (ATSDR, 1999). In general the symptoms reported in these case reports could also be due to irritancy mediated by formaldehyde rather than to the induction of immunologic mechanisms.
Further, the formation of formaldehyde-specific IgE antibodies in formaldehyde-exposed subjects has been investigated in several studies. The results did not provide evidence for formaldehyde-induced respiratory allergy. However, there was some evidence for formation of specific IgE in children exposed to low levels of formaldehyde in indoor air (ATSDR, 1999). Doi *et al.* (2003), examined 122 asthmatic and 33 non-allergic children. Formaldehyde-specific IgE was detected in two asthmatics (IgE level of 0.42 and 0.46

UA/ml), one of which had severe asthma and frequent symptoms of mucosal irritation, the other mild asthma and rare symptoms of mucosal irritation. Based on this it seems unlikely that formaldehyde is an important allergen in childhood asthma.

4.1.12.3.3 Further data on sensitisation

An anaphylactic shock (immediate systemic type of allergy mediated by IgE) after accidental i.v. application of formaldehyde during haemodialysis due to formaldehyde remaining in the system after disinfection has been described as case report (WHO, 1989). No data were given on the amount of formaldehyde applied. Mouse model experiments (conducted by Fujii *et al.*, 2005) point to some evidence that low levels of inhaled formaldehyde might enhance the sensitisation potency of other compounds in skin (inhalative exposure against 0.2 ppm formaldehyde in a mouse model for allergic contact hypersensitivity against 2,4,6-trinitrochlorobenzene).

Summary of human data

Acute toxicity:
In human case reports, local effects in the gastrointestinal tract have been reported after oral exposure. Lung function was reported to be affected by formaldehyde concentrations of 1-2 ppm at workplaces.

Skin irritation:
According to WHO, 1989, in humans, a single occlusive application of 1% formaldehyde in water will produce an irritant response in approximately 5% of the population.

Eye irritation:
Likely to be the most sensitive endpoint in humans. Odor detection in most humans well below 1 ppm. In a group of 50 subjects the 50-percentile detection threshold was 0.145 ppm, the 10-percentile detection threshold 0.020 ppm and the 90-percentile threshold 0.5 ppm (WHO, 1989). Eye irritation might occur in susceptible individuals at 0.25 ppm but significant increases in eye irritation effects are expected only at ≥1 ppm. Long-term exposure of subjects prior to the test did not alter the results in comparison to unexposed subjects; no differences between asthmatics and healthy volunteers observed (DFG, 2000). Significant nose and throat irritation occurs at dose levels of ≥2 ppm (DFG, 2000; WHO, 1989).

Skin sensitisation:
A threshold concentration for induction has been estimated to be less than 5%. In clinical (i.e., selected) patch-tested populations approximately 2% show a positive reaction to 1% formaldehyde in water and a threshold value for the elicitation of sensitised individuals has been suggested at 30 ppm (0.003%) in aqueous solution and 60 ppm (0.006%) for products containing formaldehyde (OECD, 2002). However, others suggested positive reactions to formaldehyde to be rare below concentrations of 0.025 – 0.05% (ATSDR, 1999).

Epidemiological data on carcinogenicity:
IARC (2004) reclassified formaldehyde from “probably carcinogenic to humans” (Group 2A) to “carcinogenic to humans” (Group 1) based on findings for nasal cancers from a large-scale epidemiologic study conducted by the US National Cancer Institute (Hauptman *et al.*, 2004). In 2012, this classification was re-affirmed by IARC after consideration of new studies and meta-analyses.

RAC (2012) evaluated all available data with special consideration of mechanistic insights and human origin. RAC concluded: “The existing evidence is not sufficient for classifying formaldehyde to category Carc. 1A according to CLP criteria because the available human evidence of carcinogenicity is not sufficient and a causal relationship has not been established between exposure to the agent and human cancer with sufficient confidence.” A positive association has, however, been observed between exposure to formaldehyde and the frequency of nasopharyngeal cancers in one industrial cohort for which a causal interpretation is considered plausible, but some uncertainties remain and chance, bias or confounding could not be ruled out with reasonable confidence. On the other hand, there is strong evidence from animals, evidence from one cohort study and some supporting evidence from case-control studies. Therefore, formaldehyde has been classified as Carc. 1B according to the CLP criteria. RAC also concluded that …"overall there is no convincing evidence of a carcinogenic effect at distant sites or via routes of exposure other than inhalation.” This latter issue remains highly debated (see section 3.3.7.2; IARC, 2012).

5. REFERENCES

Albert RE, Sellakumar AR, Laskin S, Kuschner M, Snyder CA (1982) Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. J. Nat. Cancer Inst. 68 (4): 5997-603, in: NTP (National Toxicology Program, 2010) Final – Report on carcinogens, Background document for Formaldehyde, 22 January 2010

Andersen DN, Sorensen H, Larsen JR, Cohr KH (2008) Survey and safety assessment of chemical substances in artificial nails and nail hardeners, Survey of Chemical Substances in Consumer Porducts, No. 95, Danish Ministry of the Environment, Environmental Protection Agency, Denmark, online: http://www2.mst.dk/udgiv/publications/2008/978-87-7052-788-0/pdf/978-87-7052-790-3.pdf

Andersen ME, Clewell HF, Bermudez E, Dodd DE, Willson GA, Campbell JL, Thomas RS (2010) Formaldehyde: integrating dosimetry, cytotoxicity, and genomics to understand dosedependent transitions for an endogenous compound. Toxicol. Sci. 118: 716-731.

Andersen ME, Clewell HF, Bermudez E, Willson GA, Thomas RS (2008) Genomic structures and dose-dependent transitions in nasal epithelial responses to inhaled formaldehyde in the rat. Toxicol. Sci. 105: 368-383.

Andersen I, Molhave L (1983) Controlled human studies with formaldehyde. In: Gibson E (ed.) Formaldehyde toxicity. Hemisphere, Washington DC, USA, pp. 154-165.

Andjelkovich D, Janszen D, Brown M, Richardson R, Miller F (1995) Mortality of iron foundry workers, IV: analysis of a subcohort exposed to formaldehyde, J. Occup. Environ. Med. 37: 826-837.

Armstrong RW, Imrey P, Lye M, Armstrong M, Yu M, Sani S (2000) Nasopharyngeal carcinoma in Malaysian Chinese: occupational exposure to particles, formaldehyde and heat. Int. J. Epidemiol. 29: 991-998.

Arts JHE, de Heer C, Woutersen R (2006) Local effects in the respiratory tract: relevance of subjectively measured irritation for setting occupational exposure limits. Int. Arch. Occup. Environ. Health 79: 283-298.

ATSDR (1999) Toxicological profile of formaldehyde. Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Public Health Service, Atlanta, Georgia, USA.

Bachand AM, Mundt KA, Mundt DJ, Montgomery RR (2010) Epidemiological studies of formaldehyde exposure and risk of leukemia and nasopharyngeal cancer: a meta-analysis. Crit. Rev. Toxicol. 40: 85-100.

Baden HP (1970) The physical properties of nail, J. Investig. Dermatol. 55: 115-122.

Ballarian C, Sarto F, Giacomelli L, Bartolucci G, Clonfero E (1992) Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. Mutat. Res. 280: 1-7.

Baran R (2002) Nail cosmetics - allergies and irritations. Am. J. Clin. Dermatol. 3: 547-555.

Baran R, Schoon D (2004) Nail fragility syndrome and ist treatment. J. Cosm. Dermatol. 3: 131-137.

Bartnik FG, Gloxhuber C, Zimmermann V (1985) Percutaneous absorption of formaldehyde in rats. Toxicol. Lett. 25: 167-172.

Basketter DA, Wright Z, Warrick E, Dearman R, Kimber I, Ryan C, Gerberick G, White I (2001) Human potency predictions for aldehydes using the local lymph node assay. Contact Dermatitis 45: 89-94.

Beane Freeman LE, Blair A, Lubin JH, Stewart PA, Hayes RB, Hoover NB, Hauptmann M (2009) Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: the National Cancer Institute Cohort. J. Natl. Cancer. Inst. 101: 751-761.

Beane Freeman LE, Blair A, Lubin JH, Stewart PA, Hayes RB, Hoover RN, Hauptmann M (2013) Mortality from solid tumors among workers in formaldehyde industries: an update of the NCI cohort, Am. J. Ind. Med. 56: 1015-1026.

Berrino F, Richiardi L, Boffetta P, Esteve J, Beletti I, Raymond L, Troschel L, Pisani P, Zubiri L, Ascunce N, Guberan E, Tuyns A, Terracini B, Merletti F (2003) Occupation and larynx and hypopharynx cancer: a job-exposure matrix approach in an international casecontrol study in France, Italy, Spain and Switzerland. Cancer Causes Control 14: 213-223.

BfR (2005) Inhalation exposure of consumers against formaldehyde. "Inhalative Exposition des Verbrauchers gegenüber Formaldehyd", paper written in German. Online: http://www.bfr.bund.de/cm/343/inhalative_exposition_des_verbrauchers_gegenueber_formaldehyd.pdf

Bhalla DK, Mahavni V, Nguyen T, McClure T (1991) Effects of acute exposure to formaldehyde on surface morphology of nasal epithelia in rats. J. Toxicol. Environ. Health 33: 171-188.

Blackburn G, Dooley JF, Schreiner C, Mackere C (1991) Specific identification of formaldehyde-mediated mutagenicity using the mouse lymphoma L5178Y+/- assay supplemented with formaldehyde dehydrogenase. In Vitro Toxicol. 4: 121-132.

Bolt HM, Morfeld P (2013) New results on formaldehyde: The 2nd International Formaldehyde Science Conference (Madrid, 19-20 April 2012). Arch. Toxicol. 87: 217-222.

Bosetti C, McLaughlin JK, Tarone RE, Pira E, LaVecchia C (2008) Formaldehyde and cancer risk: a quantitative review of cohort studies through 2006. Ann. Oncol. 19: 29-43.

Bouraoui S, Mougou S, Brahem A, Tabka F, Ben KH, Harrabi I, Mrizek N, Elghezal H, Saad A (2013) A combination of micronucleus assay and fluorescence *in situ* hybridization analysis to evaluate the genotoxicity of formaldehyde. Arch. Environ. Contam. Toxicol. 64: 337-344.

Burgaz S, Cakmak G, Erdem O, Yilmaz M, Karakaya AE (2001) Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. Neoplasma 48: 144-147; In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Buss J, Kuschinsky K, Kewitz H, Koransky W (1964) Enterale Resorption von Formaldehyd. Naunyn Schmiedebergs Arch. Exp. Pathol. Pharmacol. 247: 380-381.

Carmines EL, Rajendran N (2008) Evidence for carbon monoxide as the major factor contributing to lower fetal weights in rats exposed to cigarette smoke. Toxicol. Sci. 102: 383-391.

Carpenter CP, Smith HF (1946) Chemical burns of the rabbit cornea. Am. J. Ophthal. 29: 1363-1372.

Casanova M, Deyo D, Heck H (1989) Covalent binding of inhaled formaldehyde to DNA in the nasal mucosa of Fischer 344 rats: analysis of formaldehyde and DNA by highperformance liquid chromatography and provisional pharmacokinetic interpretation. Fundam. Appl. Toxicol. 12: 397-417.

Casanova M, Heck H (1987) Further studies on the metabolic incorporation and covalent binding of inhaled 3H- and 14C-formaldehyde in Fischer-344 rats: effect of glutathione. Toxicol. Appl. Pharmacol. 89: 105-121.

Casanova M, Heck, H, Everitt J, Harrington W, Popp J (1988) Formaldehyde concentrations in the blood of Rhesus monkeys after inhalation exposure. Food Chem. Toxic. 26: 715-716.

Casanova M, Morgan K, Gross E, Moss O, Heck H (1994) DNA-protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. Fundam. Appl. Toxicol. 23: 525-536.

Casanova M, Morgan K, Steinhagen W, Everitt J, Popp J, Heck H (1991) Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of Rhesus monkeys: pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. Fundam. Appl. Toxicol. 17: 409-428.

Casanova-Schmitz M, David R, Heck H (1984) Oxidation of formaldehyde and acetaldehyde by NAD^+-dependent dehydrogenases in rat nasal mucosal homogenates. Biochem. Pharmacol. 33: 1137-1142.

Cascieri TC, Clary JJ (1992) Formaldehyde – oral toxicity assessment. Toxicology 4: 295-304.

Cassee FR, Groten JP, Feron VJ (1996) Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde. Fundam. Appl. Toxicol. 29: 208-218.

CEFIC (2012a) The fate of formaldehyde in rats after single exposure by inhalation, Report, V20017, Study Director: Kleinnijenhuis AJ, TNO Triskelion, Zeist, The Netherlands for CEFIC, Brussels, Belgium, 03 February 2012.

CEFIC (2012b) Development of analytical methods for the analysis of formaldehyde in blood, Report, V9098, Project Manager: Kleinnijenhuis AJ, TNO Triskelion, Zeist, The Netherlands for CEFIC, Brussels, Belgium, 19 July 2011.

Chang JC, Gross E, Swenberg J, Barrow C (1983) Nasal cavity deposition, histopathology, and cell proliferation after single or repeated formaldehyde exposure in B6C3F1 mice and F344 rats. Toxicol. Appl. Pharmacol. 68: 161-176.

Checkoway H, Boffetta P, Mundt DJ, Mundt KA (2012) Critical review and synthesis of the epidemiologic evidence on formaldehyde exposure and risk of leukaemia and other lymphohematopoietic malignancies. Cancer Causes Control 23:1747-1766.

Checkoway H, Boffetta P, Mundt DJ, Mundt KA (2013) Response letter to the editor RE: Formaldehyde and leukemia: missing evidence, Cancer Causes Control 24: 205.

Chiazze L, Watkins D, Fryar C (1997) Historical cohort mortality study of a continuous filament fiberglass manufacturing plant, I: white men. J. Occup. Environ. Med. 39: 432-441.

Chow ET, Avolio AM, Lee A, Nixon R (2013) Frequency of positive patch test reactions to preservatives: The Australian experience., Australian J. Dermatol. 54: 31-35.

CIIT Chemical Industry Institute of Toxicology (1981) Final report on a chronic inhalation toxicology study in rats and mice exposed to formaldehyde. CIIT docket #10922. Colombus, OH: Battelle Colombus Laboratories.

Clayton (1999) Industrial hygiene assessment of toluene and formaldehyde concentrations in California Nail and Full Service Salons, Summary Report, Clayton Envirnmental Consultants (Clayton), Clayton Project No. 80-97276.00, 16 March 1999.

Coggon D, Harris EC, Poole J, Palmer K (2003) Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. J. Natl. Cancer Inst. 95: 1608-1615.

COLIPA TNO Consumer Inhalation Exposure Study (2008) Potential consumer inhalation exposure to airborne formaldehyde emitted from cosmetic products: Study product

information, Nail hardener, Trind Nail Repair Natural, batch 602, TNO Report V7007/02, Zeist, The Netherlands, 17 January 2008, unpublished data.

Collins JJ, Lineker GA (2004) A review and meta-analysis of formaldehyde exposure and leukaemia. Regul. Toxicol. Pharmacol. 40: 81-91.

Collins JJ, Ness R, Tyl RW, Drivanek N, Esmen NA, Hall TA (2001) A review of adverse pregnancy outcomes and formaldehyde exposure in human and animal studies. Regul. Toxicol. Pharmacol. 34: 17-34.

Collins JJ, Acquavella JF, Esmen NA (1997) An updated meta-analysis of formaldehyde exposure and upper respiratory tract cancers. J. Occup. Environ. Med. 39: 639-651.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ (2003) Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. Toxicol. Sci. 75: 432-447.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ (2004) Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. Toxicol. Sci. 82: 279-296.

Cosma GN, Marchok A (1988) Benzo[*a*]pyrene- and formaldehyde-induced DNA damage and repair in rat tracheal epithelial cells. Toxicology 51: 309-320.

Costa S, Coelho P, Costa C, Silva S, Mayan O, Santos LS, Gaspar J, Teixeira JP (2008) Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. Toxicology 252: 40-48.

CRC (2001) Physical constants of organic compounds-Formaldehyde, Lide DR (ed.), CRC Handbook of Chemistry and Physiks, 82th ed., CRC Press, Boca Raton, USA, pp. 3-166.

Creme (2013a) Formaldehyde Exposure Project – Aggregate Exposure to Formaldehyde from Cosmetics, Creme Global, Dublin, Ireland, 15 October 2013.

Creme (2013b) Formaldehyde Exposure Project – Exposure to Formaldehyde in Food and Medications, Creme Global, Dublin, Ireland, 10 October 2013.

Dalbey WE (1982) Formaldehyde and tumors in hamster respiratory tract. Toxicology 24: 9-14.

Dallas CE, Scott M, Ward J (1992) Cytogenetic analysis of pulmonary lavage and bone marrow cells of rats after repeated formaldehyde inhalation. J. Appl. Toxicol. 12: 199-203.

DeGroot A, White IR, Flyvholm MA, Lensen G, Coenraads PJ (2010b) Formaldehyde releasers in cosmetics: relationship to formaldehyde contact allergy, Part 2. Patch test relationship to formaldehyde contact allergy, experimental provocation tests, amount

of formaldehyde released, and assessment of risk to consumers allergic to formaldehyde, Contact Dermatitis 62: 18-31.

DeGroot AC, Flyvholm MA, Lensen G, Menne T, Coenraads PJ (2009) Formaldehyde releasers: relationship to formaldehyde contact allergy. Contact allergy to formaldehyde and inventory of formaldehyde-releasers. Contact Dermatitis 61: 63-85.

DeGroot AC, White IR, Flyvholm MA, Lensen G, Coenraads PJ (2010a) Formaldehyde releasers in cosmetics: Relationsship to formaldehyde contact allergy, Part 1, Characterization, frequency and relevance of sensitisation, and frequency of use in cosmetics. Contact Dermatitis 62: 2-17.

DeWit FS, DeGroot AC, WEyland JW, Bos JD (1988) An outbreak of contact dermatitis from toluene sulfonamide formaldehyde resin in a nail hardener. Contact Dermatitis 18: 280-283.

DFG (2000) Deutsche Forschungsgemeinschaft, Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, Formaldehyd, MAK Kommission zur Prüfung gesundheitlicher Arbeitsstoffe, Greim H (ed.), pp. 1-40.

Doi S, Suzuki S, Morishita M, Yamada M, Kanda Y, Torii S, Sakamoto T (2003) The prevalence of IgE sensitisation to formaldehyde in asthmatic children. Allergy 58: 668-671.

Duong A, Steinmaus C, McHale CM, Vaughan CP, Zhang L (2011) Reproductive and developmental toxicity of formaldehyde: a systematic review. Mutat. Res. 728: 118-138.

EC Regulation No. 1223/2009 of the European Parliament and of the Council of November 2009 on cosmetic products (recast, 22.12.2009) Online: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF

ECHA (2012a) Committee for Risk Assessment, RAC, Opinion proposing harmonized classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLHO-0000003155-80-01/F, 30 November 2012.

ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

ECHA (2012c) Committee for Risk Assessment, RAC, Annex 2, Responses to comments document (RCOM) to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A2, 28 November 2012.

EFSA (2014) Endogenous formaldehyde turnover in humans compared with exogenous contribution from food sources. European Food Safety Authority, Parma, Italy. Online: http://www.efsa.europa.eu/de/efsajournal/doc/3550.pdf

Elci OC, Akpinar-Elci M, Blair A, Dosemeci M (2003) Risk of laryngeal cancer by occupational chemical exposure in Turkey. J. Occup. Environ. Med. 45: 1100-1106.

Emri G, Schaefer D, Held B, Herbst C, Zieger W, Horkay I, Bayerl C (2004) Low concentrations of formaldehyde induce DNA damage and delay DNA repair after UV irradiation in human skin cells. Exp. Dermatol. 13: 305-315. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Feron VJ, Bruyntjes JP, Woutersen RA, Immel HR, Appelman LM (1988) Nasal tumors in rats after short-term exposure to a cytotoxic concentration of formaldehyde. Cancer Lett. 39: 101-111. In: Schulte A, Bernauer U, Madle S, Mielke H, Herbst U, Richter-Reichhelm HB, Appel KE, Gundert-Remy U (2006) Assessment of the Carcinogenicity of Formaldehyde [CAS No. 50-00-0], BfR (Bundesinstitut für Risikobewertung), Berlin.

Fleckman P, Allan C (2001) Surgical anatomy of the nail unit. Dematol. Surg. 27: 257-260.

Flyvholm MA, Hall BM, Agner T, tiedemann E, Greenhill P, Vanderveken W, Freeberg FE, Menne T (1997) Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients. Relationship to repeated open application test with a product containing formaldehyde release. Contact Dermatitis 36: 26-33.

Fox CH, Johnson FB, Whiting J, Roller PP (1985) Formaldehyde fixation. J. Histochem. Cytochem. 33: 845-853.

Fransway AF, Zug KA, Belsito DV, Deleo VA, Fowler JF Jr, Maibach HI, Marks JG, Mathias CGT, Pratt MD, Rietschel RL, Sasseville D, Storrs FJ, Taylor JS, Warshaw EM, Dekoven JZ (2013) North American Contact Dermatitis Group patch test results for 2007-2008. Dermatitis 24: 10-21.

Fujii K, Tsuji K, Matsuura H, Okazaki F, Takahashi S, Arata J, Iwatsuki K (2005) Effects of formaldehyde gas exposure in a murine allergic contact hypersensitivity model. Immunopharmacol. Immunotoxicol. 27: 163-175.

Galloway SM, Bloom AD, Resnick M, Margolin BH, Nakamura F, Archer P, Zeiger E (1985) Development of a standard protocol for *in vitro* cytogenetic testing with Chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. Environ. Mutagen 7: 1-51.

Gentry PR, Rodricks JV, Turnbull D, Bachand A, von Landingham C, Shipp AM, Albertini RJ, Irons R (2013) Formaldehyde and leukemia: critical review and reevaluation of the

results from a study that is the focus for evidence of biological plausibility. Crit. Rev. Toxicol. 43: 1-10.

Gettings SD, Howes D, Walters KA (1998) Experimental design considerations and use of *in vitro* skin penetration data in cosmetic risk assessment. In: Roberts MS & Walters KA (eds.) Dermal Absorption and Toxicity Assessment. Marcel Dekker Inc., Drugs and the Pharmaceutical Science. Vol. 91, chap. 20, pp. 459-487.

Gibson JE (1984) Coordinated toxicology: an example study with formaldehyde. Concepts Toxicol. 1: 276–282.

Golden R, Pyatt D, Shields PG (2006) Formaldehyde as a potential human leukemogen: an assessment of biological plausibility. Crit. Rev. Toxicol. 36: 135-153.

Gottschling LM, Beaulieu HJ, Melvin WW (1984) Monitoring of formic acid in urine of humans exposed to low levels of formaldehyde. Am. Ind. Hyg. Assoc J. 45: 19-23.

Grafstroem RC, Hsu IH, Harris C (1993) Mutagenicity of formaldehyde in Chinese hamster lung fibroblasts: synergy with ionizing radiation and *N*-nitroso-*N*-methylurea. Chem. Biol. Interact. 86: 41-49.

Halko LL (2008) Formalin containing nail care products, personal communication, Halko LL, Greenberg Traurig, 29 May 2008.

Hall A, Harrington J, Aw T (1991) Mortality study of British pathologists. Am. J. Ind. Med. 20: 83–89.

Hansch C, Leo A, Hoekman D (1995) Octanol LogP, Exploring OSAR. Hadorphobic, electronic, and steric constants, American Chemical Society (ACS), Washington, USA, 3 and 207.

Hansen JM, Contreras KM, Harris C (2005) Methanol, formaldehyde, and sodium formate exposure in rat and mouse conceptuses: a potential role of the visceral yolk sac in embryotoxicity. Birth Defects Res. A Clin. Mol. Teratol. 73: 72–82.

Hansen J, Olsen J (1995) Formaldehyde and cancer morbidity among male employees in Denmark. Cancer Causes Control 6: 354-360.

Hauptmann M, Lubin J, Stewart P, Hayes R, Blair A (2003) Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J. Natl. Cancer Inst. 95: 1615-1623.

Hauptmann M, Lubin J, Stewart P, Hayes R, Blair A (2004) Mortality from solid cancers among workers in the formaldehyde industries. Am. J. Epidemiol. 159: 1117-1130.

Hauptmann M, Stewart PA, Lubin JH, Beane Freeman LE, Hornung RW, Herrick RF, Hoover RN, Fraumeni JF, Jr, Blair A, Hayes RB (2009) Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde, J. Natl. Cancer Inst. 101: 1696-1708.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983) *Salmonella* mutagenicity test results for 250 chemicals. Environ. Mutagen. Suppl 1: 1-142.

Hayes R, Blair A, Stewart P, de Bruyn A, Gerin M (1990) Mortality of U.S. embalmers and funeral directors, Am. J. Ind. Med. 18. 641-652.

He JL, Jin LF, Jin HY (1998) Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. Biomed. Environ. Sci. 11: 87-92.

Heck H, Chin TY, Casanova-Schmitz M (1983) Distribution of [^{14}C]formaldehyde in rats after inhalation exposure. Gibson E (ed.) Formaldehyde toxicity. Hemisphere, Washington DC, USA, pp. 26-37.

Heck HA, Casanova M (2004) The implausibility of leukaemia induction by formaldehyde: a critical review of the biological evidence on distant site toxicity. Regul. Toxicol. Pharmacol. 40: 92-106.

Heck HA, Casanova-Schmitz M, Dodd P, Schachter E, Witek T, Tosun T (1985) Formaldehyde (CH_2O) concentrations in the blood of humans and Fischer-344 rats exposed to CH_2O under controlled conditions. Am. Ind. Hyg. Assoc. J. 46: 1-3.

Hedberg JJ, Hoog JO, Grafstrom RC (2002) Assessment of formaldehyde metabolizing enzymes in human oral mucosa and cultured oral keratinocytes indicate high capacity for detoxification of formaldehyde. In: Crucial Issues in Inhalation Research — Mechanistic, Clinical and Epidemiologic (INIS Monographs). Heinrich U, Mohr U, editors. Stuttgart: Fraunhofer IRB Verlag, pp. 103-115.

Hildesheim A, Dosemeci M, Chan CC, Chen CJ, Cheng YJ, Hsu MM, Chen IH, Mittl B, Sun B, Levine P, Chen JY, Brinton L, Yang CS (2001) Occupational exposure to wood, formaldehyde, and solvents and risk of nasopharyngeal carcinoma. Cancer Epidemiol. Biomarkers Prevent. 10: 1145-1153.

Hilton J, Dearman R, Basketter D, Scholes E, Kimber I (1996) Experimental assessment of the sensitising properties of formaldehyde. Food Chem. Toxicol. 34: 571-578.

Huldin DH (1968) Hemorrhages of the lips sdecondary to nail hardeners. Cutis 4: 709-711.

IARC (1995) IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans, Volume 62, Wood dust and formaldehyde, Lyon, Farnce, 217-365. Online: http://monographs.iarc.fr/ENG/Monographs/vol62/mono62-7.pdf

IARC (2006) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 88 Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol, Lyon, France, 40-325. Online: http://monographs.iarc.fr/ENG/Monographs/vol88/mono88-6.pdf

IARC (2012) IARC Monograph 100F, Chemical Agents and Related Occupations, pp. 401-435. Online: http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-29.pdf

Iorizzo M, Pazzaglia M, Piraccini BM, Tullo S, Tosti A (2004) Brittle nails. J. Cosmetic Dermatol. 3: 138-144.

IPCS (2002) Concise International Chemical Assessment Document No 40: Formaldehyde. WHO, Geneva, Switzerland, pp. 1-75. Online: http://www.inchem.org/documents/cicads/cicads/cicad40.htm

Iversen OH (1986) Formaldehyde and skin carcinigenesis. Environ. Int. 12: 541-544.

Jackson EM (2008a) personal communication with results of 40 patch tested patients, 18 November 2008.

Jackson EM (2008b) Formaldehyde sensitisation: A critical review of the contact dermatitis literature, Jackson EM, Jackson Research Associates, Sumner, Washington, USA prepared for the Nail Manufacturers Council, Scottsdale, Arizona, USA, 25 January 2008.

Jakab MG, Klupp T, Besenyei K, Biro A, Major J, Tompa A (2010) Formaldehyde-induced chromosomal aberrations and apoptosis in peripheral blood lymphocytes of personnel working in pathology departments. Mutat. Res. 698: 11-17.

Jeffcoat AR, Chasalow F, Feldman D, Marr H (1983) Disposition of ^{14}C-formaldehyde after topical exposure to rats, guinea pigs, and monkeys. In: Gibson JE (ed.) Formaldehyde toxicity, Hemisphere Publishing Corporation, Washington DC, pp. 38-50.

Johannsen FR, Levinskas GJ, Tegeris AS (1986) Effects of formaldehyde in the rat and dog following oral exposure. Toxicol. Lett. 30: 1-6.

JORF (2011) n°0281 du 4 décembre 2011 page 20529 - texte n° 4 – DECRET Décret n° 2011 - 1727 du 2 décembre 2011 relatif aux valeurs-guides pour l'air intérieur pour le formaldéhyde et le benzene (i.e., related to formaldehyde and benzene guided value for indoor air). Online: http://www.developpement-durable.gouv.fr/Valeurs-guides-de-l-air-interieur.html

Kamata E, Nakadate M, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M, Kurokawa Y (1997) Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J. Toxicol. Sci. 22: 239-254.

Kamber M, Fluckiger-Isler S, Engelhardt G, Jaeckh R, Zeiger E (2009) Comparison of the Ames II and traditional Ames test responses with respect to mutagenicity, strain specificities, need for metabolism and correlation with rodent carcinogenicity. Mutagenesis 24: 359-366. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Keller DA, Heck H, Randall H, Morgan K (1990) Histochemical localization of formaldehyde dehydrogenase in the rat. Toxicol. Appl. Pharmacol. 106: 311-326.

Kelly T, Smith DL, Satola J (1999) Emission rates of formaldehyde from materials and consumer products found in California Homes. Environ. Sci. Technol. 33: 81-88.

Kerns WD, Pavkov K, Donofrio D, Gralla E, Swenberg J (1983) Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res. 43: 4382-4392.

Kimber I, Hilton J, Botham P, Basketter D, Scholes E, Miller K, Robbins M, Harrison P, Gray T, Waite S (1991) The murine local lymph node assay: results of an inter-laboratory trial. Toxicol. Lett. 55: 203-213.

Kimbell JS, Overton JH, Subramaniam RP, Schlosser PM, Morgan KT, Conolly RB, Miller FJ (2001b) Dosimetry modeling of inhaled formaldehyde: binning nasal flux predictions for quantitative risk assessment. Toxicol. Sci. 64: 111-121.

Kimbell JS, Subramaniam RP, Gross EA, Schlosser PM, Morgan KT (2001a) Dosimetry modeling of inhaled formaldehyde: comparisons of local flux predictions in the rat, monkey, and human nasal passages. Toxicol. Sci. 64: 100-110.

Kleinnijenhuis AJ, Staal YCM, Duistermaat E, Engel R, Woutersen RA (2013) The determination of exogenous formaldehyde in blood of rats during and after inhalation exposure. Food Chem. Toxicol. 52: 105-112.

Kligerman AD, Phelps M, Erexsen G (1984) Cytogenetic analysis of lymphocytes from rats following formaldehyde inhalation. Toxicol. Lett. 21: 241-246. In: Kochhar R, Nanda V, Nagi B, Mehta S (1986) Formaldehyde-induced corrosive gastric cicatrisation: case report. Human Toxicol. 5: 381-382.

Knasmueller S, Holland N, Wultsch G, Jandl B, Burgaz S, Misik M, Nersesyan A (2011) Use of nasal cells in micronucleus assays and other genotoxicity studies. Mutagenesis 26: 231-238.

Kochhar R, Nanda V, Nagi B & Mehta S (1986) Formaldehyde-induced corrosive gastric cicatrisation: case report. Human Toxicol. 5: 381-382.

Kuehner S, Holzmann K, Speit G (2013) Characterization of formaldehyde's genotoxic mode of action by gene expression analysis in TK6 cells. Arch. Toxicol. 87: 1999-2012.

Kuehner S, Schlaier M, Schwarz K, Speit G (2012) Analysis of leukemia-specific aneuploidies in cultured myeloid progenitor cells in the absence and presence of formaldehyde exposure. Toxicol. Sci. 128: 72-78.

Kulle TJ (1993) Acute odor and irritation response in healthy nonsmokers with formaldehyde exposure. Inhal. Toxicol. 5: 323-332.

Kulle TJ, Sauder L, Hebel J, Green D, Chatham M (1987) Formaldehyde dose-response in healthy nonsmokers, J. Air Pollut. Control Assoc. 37: 919-924.

Kuper FC (2007) Formaldehyde and NALT. Formaldehyde International Science Conference, Barcelona 20-21 September 2007. In: ECHA (2012a) Committee for Risk Assessment,

RAC, Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/F, 30 November 2012.

Kuper FC (2012) Local lymphoid tissues and formaldehyde. 2nd International Formaldehyde Science Conference, 19-20 April 2012, Madrid. In: ECHA (2012a) Committee for Risk Assessment, RAC, Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/F, 30 November 2012.

Kuper FC, van OL, Ma-Hock L, Durrer S, Woutersen RA (2011) Hyperplasia of the lymphoepithelium of NALT in rats but not in mice upon 28-day exposure to 15 ppm formaldehyde vapor. Exp. Toxicol. Pathol. 63: 25-32.

Ladeira C, Viegas S, Carolino E, Prista J, Gomes MC, Brito M (2011) Genotoxicity biomarkers in occupational exposure to formaldehyde – the case of histopathology laboratories. Mutat. Res. 721: 15-20.

Laforest L, Luce D, Goldberg P, Begin D, Gerin M, Demers P, Brugere J, Leclerc A (2000) Laryngeal and hypopharyngeal cancers and occupational exposure to formaldehyde and various dusts: a case-control study in France. Occup. Environ. Med. 57: 767-773.

Lang I, Bruckner T, Triebig G (2008) Formaldehyde and chemosensory irritation in human: a controlled human exposure study. Regul. Toxicol. Pharmacol. 50: 23-36.

Lefebvre MA, Meuling WJA, Engel R, Coroama MC, Renner G, Pape W, Nohynek GJ (2012) Consumer inhalation exposure to formaldehyde from use of personal care products/cosmetics. Regulat. Toxicol. Pharmacol. 63: 171-176.

Levine R, Andjelkovich D, Shaw L (1984) The mortality of Ontario undertakers and a review of formaldehyde-related mortality studies. J. Occup. Med. 26: 740-746.

Liber HL, Benforado K, Crosby RM, Simpson D, Skopek TR (1989) Formaldehyde-induced and spontaneous alterations in human HPRT DNA sequence and mRNA expression. Mutat. Res. 226: 31-37.

Lin D, Guo Y, Yi J, Kuang D, Li X, Deng H, Huang K, Guan L, He Y, Zhang X, Hu D, Zhang Z, Zheng H, Zhang X, McHale C, Zhang L, Wu T (2013) Occupational Exposure to formaldehyde and genetic damage in the peripheral blood lymphocytes of plywood workers. J. Occup. Health 55: 284-291.

Liu Y, Li CM, Lu Z, Ding S, Yang X, Mo J (2006) Studies on formation and repair of formaldehyde-damaged DNA by detection of DNA-protein crosslinks and DNA breaks. Front Biosci. 11: 991-997. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Liu YR, Zhou Y, Qiu W, Zeng JY, Shen LL, Li AP, Zhou JW (2009) Exposure to formaldehyde induces heritable DNA mutations in mice. J. Toxicol. Environ. Health 72: 767-773.

Loden M (1986) The *in vitro* permeability of human skin to benzene, ethylene glycol, formaldehyde, and *n*-hexane. Acta Pharmacol. Toxicol. 58: 382-389.

Logue JN, Barrick MK, Jessup GL Jr (1986) Mortality of radiologists and pathologists in the Radiation Registry of Physicians. J. Occup. Med. 28: 91-99.

Lu K, Collins LB, Ru H, Bermudez E, Swenberg JA (2010). Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. Toxicol. Sci. 116: 441-451.

Lu K, Ye W, Gold A, Ball LM, Swenberg JA (2009) Formation of *S*-[1-(N^2-deoxyguanosinyl)methyl]glutathione between glutathione and DNA induced by formaldehyde. J. Am. Chem. Soc. 131: 3414-3415. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonized classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLHO-0000003155-80-01/A1, 28 November 2012.

Lu K, Moeller BC, Doyle-Eisele M, McDonald J, Swenberg JA (2011) Molecular dosimetry of N^2-hydroxymethyl-dG DNA adducts in rats exposed to formaldehyde. Chem. Res. Toxicol. 24: 159-161.

Luce D, Leclerc A, Begin D, Demers P, Gerin M, Orlowski E, Kogevinas M, Belli S, Bugel I, Bolm-Audorf U, Brinton L, Comba P, Hardell L, Hayes R, Magnani C, Merler E, Preston-Martin S, Vaughan T, Zheng W, Boffetta P (2002) Sinonasal cancer and occupational exposures: a pooled analysis of 12 case-control studies. Cancer Causes Control 13: 147-157.

Luce D, Gérin M, Leclerc A, Morcet JF, Brugere J, Goldberg M (1993) Sinonasal cancer and occupational exposure to formaldehyde and other substances. Int. J. Cancer 53: 224-231.

Mackere CR, Angelosanto FA, Blackburn GR, Schreiner CA (1996) Identification of formaldehyde as the metabolite responsible for the mutagenicity of methyl tertiary-butyl ether in the activated mouse lymphoma assay. Proc. Soc. Exp. Biol. Med. 212: 338-341. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Mahboubi A, Koushik A, Siemiatycki J, Lavoue J, Rousseau MC (2013) Assessment of the effect of occupational exposure to formaldehyde on the risk of lung cancer in two Canadian population-based case-control studies, Scand. J. Work Environ. Health 39: 401-410.

Malek FA, Möritz KU, Fanghänel J (2003a) A study on the effects of inhalative formaldehyde exposure on water labyrinth test performance in rats. Ann. Anat. 185: 277-285.

Malek FA, Möritz KU, Fanghänel J (2003b) A study on specific behavioural effects of formaldehyde in the rat. J. Exp. Animal Sci. 43: 160-170.

Marks TA, Worthy W, Staples R (1980) Influence of formaldehyde and Sonacide® (potentiated acid glutaraldehyde) on embryo and fetal development in mice. Teratology 22: 51-58.

Marnett LJ, Hurd H, Hollstein M, Levin D, Esterbauer H, Ames BN (1985) Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. Mutat. Res. 148: 25-34.

Maronpot RR, Miller R, Clarke W, Westerberg R, Decker J, Moss O (1986) Toxicity of formaldehyde vapour in B6C3F1 mice exposed for 13 weeks. Toxicology 41: 253-266.

Marsh GM (2010) Comments on the recommendation from the expert panel report (part B) on formaldehyde, 08 February 2010.

Marsh GM, Youk A, Buchanich J, Cassidy L, Lucas L, Esmen N, Gathuru I (2002) Pharyngeal cancer mortality among chemical plant workers exposed to formaldehyde. Toxicol. Ind. Health 18: 257-268.

Marsh GM, Youk AO (2005) Reevaluation of mortality risks from nasopharyngeal cancer in the formaldehyde cohort study of the National Cancer Institute. Regul. Toxicol. Pharmacol. 42: 275-283.

Marsh GM, Youk AO, Buchanich JM, Erdal S, Esmen NA (2007a) Work in the metal industry and nasopharyngeal cancer mortality among formaldehyde-exposed workers. Regul. Toxicol. Pharmacol. 48: 308-319.

Marsh GM, Youk AO, Morfeld P (2007b) Mis-specified and non-robust mortality risk models for nasopharyngeal cancer in the National Cancer Institute formaldehyde worker cohort study. Regul. Toxicol. Pharmacol. 47: 59-67.

Martin WJ (1990) A teratology study of inhaled formaldehyde in the rats. Reprod. Toxicol. 4: 237-239.

Marzulli F, Maguire HC (1982) Usefulness and limitations of various guinea-pig test methods in detecting human skin sensitizers — validation of guinea-pig tests for skin hypersensitivity. Food Chem. Toxicol. 20: 67-74.

Marzulli FN, Maibach HI (1974) The use of graded concentrations in studying skin sensitizers: experimental contact sensitisation in man. Food Cosmet. Toxicol. 12: 219-227.

Mavala SA (1964) Clinic expertise of Mavala Scientific nail hardener (MSNH), Mavala SA Geneve, Switzerland, Original French Report A la demande des Etablissements C. David-Rabot, 49 rue de Bitche, à Courbevoie (Seine), Nous avons étudié la tolérance

et la valeur thérapeutique d'une solution dénommée "Mavala" dans le présent rapport et dont la formule est la suivante, C.E.D.A., Centre D'Etudes Dermatologiques et Alergogiques, Paris, France, 29 January 1964.

Mavala SA (2009) Mavala Scientific Penetrating Nail Hardener, Product desciption including use and safety instructions, Mavala, Geneva, Switzerland.

Mavala SA (2010) Mavala Scientifique Nail Hardener, Determination of the applied amount per application (10) nails, Perrenot B and Maute J, Mavala International SA, Geneve, Switzerland, 19 March 2010.

Mavala SA, TRIND Cosmetics B.V. (2013) Lack of alternatives for formaldehyde used in nail hardeners.

Meng F, Bermudez E, McKinzie PB, Andersen ME, Clewell HJ, III, Parsons (2010) Measurement of tumor-associated mutations in the nasal mucosa of rats exposed to varying doses of formaldehyde. Regul. Toxicol. Pharmacol. 57: 274-283.

Merck (1996) The Merck Index, Budavari S (ed.), 12th edition, Merck & Co, Inc Whitehouse station, NJ, USA, 717-718.

Merck O, Speit G (1998) Significance of formaldehyde-induced DNA-protein crosslinks for mutagenicity. Environ. Mol. Mutagen. 32: 260-268.

Migliore L, Ventura L, Barale R, Loprieno N, Castellino S, Pulci R (1989) Micronuclei and nuclear anomalies induced in the gastro-intestinal epithelium of rats treated with formaldehyde. Mutagenesis 4: 327-334.

Mitchel JC (1981) Non-inflammatory onycholysis from formaldehyde-containing nail hardener. Contact Dermatitis 7: 173.

Miyachi T, Tsutsui T (2005) Ability of 13 chemical agents used in dental practice to induce sister-chromatid exchanges in Syrian hamster embryo cells. Odontology 93: 24-29. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Moeller BC, Lu K, Doyle-Eisele M, McDonald J, Gigliotti A, Swenberg JA (2011) Determination of N^2-hydroxymethy-dG adducts in the nasal epithelium and bone marrow of nonhuman primates following ^{13}C-formaldehyde inhalation exposure. Chem. Res. Toxicol. 24: 162-164.

Monticello TM, Miller F, Morgan K (1991) Regional increases in rat nasal epithelial cell proliferation following acute and subchronic inhalation of formaldehyde. Toxicol. Appl. Pharmacol. 111: 409-421.

Monticello TM, Swenberg J, Gross E, Leininger J, Kimbell J, Seilkop S, Starr T, Gibson J, Morgan K (1996) Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res. 56: 1012-1022.

Morfeld P (2013) Formaldehyde and leukemia: missing evidence. Cancer Causes Control 24: 203-204.

Morita T, Asano N, Awogi T, Sasaki Y, Sato S, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, Hayashi M (1997) Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B): The summary report of the 6th collaborative study by CSGMT/JEMS MMS. Collaborative Study of the Micronucleus Group Test. Mammaliam Mutagenicity Study Group. Mutat. Res. 389: 3-122.

Musak L, Smerhovsky Z, Halasova E, Osian O, Letkova L, Vodickova L, Polakova V, Buchancova J, Hemminki K, Vodicka P (2013) Chromosomal damage among medical staff occupationally exposed to volatile anesthetics, antineoplatic drugs and formaldehyde. Scand. J. Work Environ. Health 39: 618-630.

Nagorny PA, Sudakova ZA, Schablenko AM (1979) On the general toxic and allergic action of formaldehyde. Gig. Tr. Prof. Zabol. 1: 27-30. (Original Reference in Russian; cited in OECD 2002.)

Neuss S, Moepps B, Speit G (2010a) Exposure of human nasal epithelial cells to formaldehyde does not lead to DNA damage in lymphocytes after co-cultivation. Mutagenesis 25: 359-364.

Neuss S, Zeller J, Ma-Hock L, Speit G (2010b) Inhalation of formaldehyde does not induce genotoxic effects in brocho-alveolar lavage (BAL) cells of rats. Mutat. Res. 695: 61-68.

NICNAS (2006) National Industrial Chemicals Notification Assessment Scheme, Priority Existing Chemical Assessment Report No. 28, Formaldehyde, Australian Government, Sydney, Australia, 1-351. Online: http://www.nicnas.gov.au/Publications/CAR/PEC/PEC28/PEC_28_Full_Report_PDF.pdf

Nielsen GD, Larsen ST, Wolkoff P (2013) Recent trend in risk assessment of formaldehyde exposures from indoor air. Arch. Toxicol. 87: 73-98.

Norton LA (1991) Common and uncommon reactions to formaldehyde-containing nail hardeners. Seminars in Dermatology 10: 29-33.

NTP (National Toxicology Program, 2010) Final – Report on carcinogens, Background document for Formaldehyde, U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC, USA, 22 January 2010. Online: http://ntp.niehs.nih.gov/ntp/roc/twelfth/2009/November/Formaldehyde_BD_Final.pdf

OECD (2002) SIDS Initial Assessment Report for SIAM 14, March 2002, Formaldehyde, ICCA documentation on formaldehyde, UNEP publications. Online:

http://www.chem.unep.ch/irptc/sids/OECDSIDS/FORMALDEHYDE.pdf

Olsen JH, Asnaes S (1986) Formaldehyde and the risk of squamous cell carcinoma of the sinonasal cavities. Br. J. Ind. Med. 43: 769-774.

Olsen JH, Jensen SP, Hink M, Faurbo K, Breum NO, Jensen OM (1984) Occupational formaldehyde exposure and increased nasal cancer risk in man. Int. J. Cancer 34: 639-644.

Orsiere T, Sari-Minodier I, Iarmarcovai G, Botta A (2006) Genotoxic risk assessment of pathology and anatomy laboratory workers exposed to formaldehyde by use of personal air sampling and analysis of DNA damage in peripheral lymphocytes. Mutat. Res. 605: 30-41.

Ott M, Teta M, Greenberg H (1989) Lymphatic and hematopoietic tissue cancer in a chemical manufacturing environment. Am. J. Ind. Med. 16: 631-643. In: NTP (National Toxicology Program, 2010) Final – Report on carcinogens, Background document for Formaldehyde, 22 January 2010.

Owen BA, Dudney CS, Tan EL, Easterly CE (1990) Formaldehyde in drinking water: comparative hazard evaluation and an approach to regulation. Regul. Toxicol. Pharmacol. 11: 220-236.

Ozen OA, Yaman M, Sarsilmaz M, Sonqur A, Kus I (2002) Testicular zinc, copper and iron concentrations in male rats exposed to subacute and subchronic formaldehyde gas inhalation. J. Trace Elem. Med. Biol. 16: 119-122.

Paget-Bailly S, Cyr D, Luce D (2012) Occupational exposures and cancer of the larynx – systematic review and meta-analysis. J. Occupat. Environ. Med. 54: 71-84.

Pala M, Ugolini D, Ceppi M, Rizzo F, Maiorana L, Bolognesi C, Schiliro T, Gilli G, Bigatti P, Bono R, Vecchio D (2008) Occupational exposure to formaldehyde and biological monitoring of Research Institute workers. Cancer Detect. Prevent. 32: 121-126. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Pandey CK, Agarwal A, Baronia A, Singh N (2000) Toxicity of ingested formalin and its management. Hum. Exp. Toxicol. 19: 360-366.

Patterson DL, Gross E, Bogdanffy M, Morgan K (1986) Retention of formaldehyde gas by nasal passages of F344 rats. Toxicologist 6: 55.

Paustenbach D, Alarie Y, Kulle T, Schachter N, Smith R, Swenberg J, Witschi H, Harowitz SB (1997) A recommended occupational exposure limit for formaldehyde based on irritation. J. Toxicol. Environ. Health 50: 217-263.

Pesch B, Pierl CB, Gebel M, Gross I, Becker D, Johnen G, Rihs HP, Donhuijsen K, Lepentsiotis V, Meier M, Schulze J, Brüning T (2008) Occupational risks for adenocarcinoma of the nasal cavity and paranasal sinuses in the German wood industry. Occup. Environ. Med. 65: 191-196.

Pontén A, Goossens A, Bruze M (2013) Recommendation to include formaldehyde 2.0% aqua in the European baseline patch test series. Contact Dermatitis 69: 372-374.

Pontén A, Aalto-Korte K, Agner T, Andersen KE, Giménez-Arnau AM, Gonçalo M, Goossens A, Johansen JD, Le Coz CJ, Maibach HI, Rustemeyer T, White IR, Bruze M (2013) Patch testing with 2.0% (0.60 mg/cm^2) formaldehyde instead of 1.0% (0.30 mg/cm^2) detects significantly more contact allergy. Contact Dermatitis 2013 68: 50-53.

Pickrell JA, Mokler BV, Griffis LC, Hobbs CH, Bathija A (1983) Formaldehyde release rate coefficients from selected consumer products. Environ. Sci. Technol. 17: 753-757.

Pinkerton LE, Hein M, Stayner L (2004) Mortality among a cohort of garment workers exposed to formaldehyde. Occup. Environ. Med. 61: 193-200.

Quievryn G, Zhitkovich A (2000) Loss of DNA-protein crosslinks from formaldehyde exposed cells occurs through spontaneous hydrolysis and an active repair process linked to proteosome function. Carcinogenesis 21: 1573-1580. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonized classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLHO-0000003155-80-01/A1, 28 November 2012.

RAC (2012) Opinion proposing harmonised classification and labelling at EU level of formaldehyde. Committee for Risk Assessment (RAC), ECHA. Online: http://echa.europa.eu/documents/10162/254a73cf-ff8d-4bf4-95d1-109f13ef0f5a

Rager JE, Moeller BC, Doyle-Eisele M, Kracko D, Swenberg JA, Fry RC (2013) Formaldehyde and epigenetic alterations: microRNA changes in the nasal epithelium of nonhuman primates. Environ. Health Perspect. 121: 339-344.

Rhein LD (2001) Nails - Review of Structure, Function and Strategies to Treat Disorders, GlaxoSmithKline, November 2001, http://www.nyscc.org/news/archive/tech1101.html

Robbins JD, Norred W, Bathija A, Ulsamer A (1984) Bioavailability in rabbits of formaldehyde from durable-press textiles. J. Toxicol. Environ. Health 14: 453-463.

Rodrigues DF, Neves DR, Pinto JM, Alves MFF, Fulgencio ACF (2012) Results of patch-tests from Santa Casa de Belo Horizonte Dermatology Clinic, Belo Horizonte, Brazil, from 2003 to 2010. Anais Brasileiros de Dermatologia 87: 800-803.

Roush GC, Walrath J, Stayner LT, Kaplan SA, Flannery JT, Blair A (1987) Nasopharyngeal cancer, sinonasal cancer, and occupations related to formaldehyde: a case-control study. J. Natl. Cancer Inst. 79: 1221-1224.

Rowland Paye CME (2004) Editorial – Brittle nails, fragile nails. Cosmetic Dermatol. 3: 119-121.

Runne U, Orfanos CE (1981) The human nail: structure, growth and pathological changes. Curr. Probl. Dermatol. 9: 102-149.

Rusch GM, Clary J, Rinehart W, Bolte H (1983) A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. Toxicol. Appl. Pharmacol. 68: 329-343.

Saillenfait AM, Bonnet P, de Ceaurriz J (1989) The effect of maternally inhaled formaldehyde on embryonal and fetal development in rats. Food Chem. Toxicol. 27: 545-548.

Salthammer T, Mentese S, Marutzky R (2010) Formaldehyde in the indoor environment. Chem. Rev. 110: 2536-2572.

Sangster J (1989) Octanol-Water Partition Coefficients of Simple Organic Compounds. J. Phys. Chem. Ref. Data 18: 1111-1229.

Sari DK, Kuwahara S, Tsukamoto Y, Hori H, Kunugita N, Arashidani K, Fujimaki H, Sasaki F (2004) Effects of prolonged exposure to low concentrations of formaldehyde on the corticotrophin releasing hormone neurons in the hypothalamus and adrenocorticotropic hormone cells in the pituitary gland in female mice. Brain Res. 1013: 107-116.

Schmid E, Göggelmann W, Bauchinger M (1986) Formaldehyde-induced cytotoxic, genotoxic and mutagenic response in human lymphocytes and *Salmonella typhimurium*. Mutagenesis 1: 427-431.

Schmid O, Speit G (2007) Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. Mutagenesis 22: 69-74.

Schnuch A, Lesmann H, Geier J, Uter W (2011) Contact allergy to preservatives. Analysis of IVDK data 1996 – 2009, Brit. J. Dermatology 164: 1316-1325.

Schnuch A, Uter W, Lessmann H, Geier J (2008) Contact allergy to preservatives. Results of the Information Network of Departments of Dermatology (IVDK) 1996 to 2007. [Kontaktallergien gegen Konservierungsmittel Ergebnisse des Informationsverbundes Dermatologischer Kliniken (IVDK) 1996 bis 2007] Allergo J. 17: 631-638.

Schulte A, Bernauer U, Madle S, Mielke H, Herbst U, Richter-Reichhelm HB, Appel KE, Gundert-Remy U (2006) Assessment of the Carcinogenicity of Formaldehyde [CAS No. 50-00-0], BfR (Bundesinstitut für Risikobewertung), Berlin, Germany. Online: http://www.bfr.bund.de/cm/238/assessment_of_the_carcinogenicity_of_formaldehyde.pdf

Schwilk E, Zhang L, Smith MT, Smith AH, Steinmaus (2010) Formaldehyde and leukemia: an updated meta-analysis and evaluation of bias. J. Occup. Environ. Med. 52: 878-886.

Sekizawa J, Yasuhara K, Suyama Y, Yamanaka S, Tobe M, Nishimura M (1994) A simple method for screening assessment of skin and eye irritation. J. Toxicol. Sci. 19: 25-35.

Sellakumar AR, Snyder CA, Solomon JJ, Albert RE (1985) Carcinogenicity of formaldehyde and hydrogen chloride in rats. Toxicol. Appl. Pharmacol. 81: 401-406.

Shaham J, Bornstein Y, Gurvich R, Rashkovsky M, Kaufman Z (2003) DNA-protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. Occup. Environ. Med. 60: 403-409.

Siew SS, Kauppinen T, Kyyronen P, Heikkila P, Pukkala E (2012) Occupational exposure to wood dust and formaldehyde and risk of nasal, nasopharyngeal, and lung cancer among Finnish men. Cancer Manag. Res. 4: 223-232.

Skog E (1950) A toxicological investigation of lower aliphatic aldehydes. Acta Pharmacol. Toxicol. 6: 299-318.

Smyth HF, Seaton J, Fischer L (1941) The single dose toxicity of some glycols and derivatives. J. Ind. Hyg. Toxicol. 23: 259-268.

Soffritti M, Belpoggi F, Lambertin L, Lauriola M, Padovani M, Maltoni C (2002) Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Ann. NY Acad. Sci. 982: 87-105.

Soffritti M, Maltoni C, Maffei F, Biagi R (1989) Formaldehyde: an experimental multipotential carcinogen. Toxiccol. Ind. Health 5: 699-730.

Speit G, Merk O (2002) Evaluation of mutagenic effects of formaldehyde *in vitro*: detection of crosslinks and mutations in mouse lymphoma cells. Mutagenesis 17: 183-187. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Speit G, Neuss S, Schmid O (2010) The human lung cell line A549 does not develop adaptive protection against the DNA-damaging action of formaldehyde. Environ. Mol. Mutagen. 51: 130-137.

Speit G, Schmid O (2006) Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells. Mutat. Res. 613: 1-9.

Speit G, Schmid O, Fröhler-Keller M, Lang I, Triebig G (2007) Assessment of local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated buccal cells. Mutat. Res. 627: 129-135.

Speit G, Schmid O, Neuss S, Schutz P (2008) Genotoxic effects of formaldehyde in the human lung cell line A549 and in primary human nasal epithelial cells. Environ. Mol. Mutagen. 49: 300-307. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification

and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Speit G, Schutz P, Hogel J, Schmid O (2007b) Characterization of the genotoxic potential of formaldehyde in V79 cells. Mutagenesis 22: 387-394.

Speit G, Zeller J, Schmid O, Elhajouji A, Ma-Hock L, Neuss S (2009) Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. Mutat. Res. 677: 76-85.

Speit G, Ladeira C, Linsenmeyer R, Schutz P, Hogel J (2012) Re-evaluation of a reported increased micronucleus frequency in lymphocytes of workers occupationally exposed to formaldehyde. Mutat. Res. 744: 161-166.

Stellman S, Demers P, Colin D, Boffetta P (1998) Cancer mortality and wood dust exposure among participants in the American Cancer Society Cancer Prevention Study-II (CPS-II). Am. J. Ind. Med. 34: 229-237.

Stroup NE, Blair A, Erikson GE (1986) Brain cancer and other causes of death in anatomists. J. Natl. Cancer Inst. 77: 1217-1224.

Sul D, Kim H, Oh E, Phark S, Cho E, Choi S, Kang HS, Kim EM, Hwang KW, Jung WW (2007) Gene expression profiling in lung tissues from rats exposed to formaldehyde. Arch. Toxicol. 81: 589-597. In: ECHA (2012b) Committee for Risk Assessment, RAC, Annex 1, Background document to the Opinion proposing harmonised classification and labelling at EU level for Formaldehyde, European Chemical Agency, CLH-O-0000003155-80-01/A1, 28 November 2012.

Svedman C, Andersen KE, Brandao FM, Bruynzeel DP, Diepgen TL, Frosch PJ, Rustemeyer T, Gimenez-Arnau A, Goncalo M, Goossens A, Johansen JD, Lahti A, Menne T, Seidenari S, Tosti A, Wahlberg JE, White IR, Wilkinson JD, Mowitz M, Bruze M (2012) Follow-up of the monitored levels of preservative sensitivity in Europe: overview of the years 2001-2008. Contact Dermatitis 67: 312-314.

Tallon M, Merianos JJ, Subramanian S (2009) Non-destructive method for determining the actual concentration of free formaldehyde in personal care formulations containing formaldehyde donors. SOFW J. 135: 22-32.

Til HP, Woutersen R, Feron V, Clary J (1988) Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking water study in rats. Food Chem. Toxicol. 26: 447-452.

Til HP, Woutersen R, Feron V, Hollanders V, Falke H, Clary J (1989) Two-years drinking water study of formaldehyde in rats. Food Chem. Toxicol. 27: 77-87.

Titenko-Holland N, Levine A, Smith M, Qiuntana P, Boeniger M, Hayes R, Suruda A, Schulte P (1996) Quantification of epithelial cell micronuclei by fluorescence *in situ*

hybridization (FISH) in mortuary science students exposed to formaldehyde. Mutat. Res. 371: 237-248.

TNO (2002) TNO Report V3847 – Development and validation of an analytical method for the determination of formaldehyde in nail hardener, TNO, Zeist, The Netherlands, 05 April 2002, unpublished data.

TNO (2003) Shelf life nail repair T13351135, TNO Report ARA 03-0582/SCHA, TNO, Zeist, The Netherlands, 28 February 2003, unpublished data.

Tobe M, Naito K, Kurokawa Y (1989) Chronic toxicity study on formaldehyde administered orally to rats. Toxicology 56: 79-86.

TRIND (2003) Analytical technical documentation of Trind Nail Repair, 07 July 2003, unpublished data.

TRIND (2006) Certificate of analysis of Trind Nail Repair batch 602, 28 March 2006, unpublished data.

TRIND (2006) Certificate of analysis of Trind Nail Repair batch 602, 28 March 2006 unpublished data.

Tsuchiya K, Hayashi Y, Onodera M, Hasegawa T (1975) Toxicity of formaldehyde in experimental animals. Kefo J. Med. 24: 19-37.

Ulker OC, Ates I, Atak A, Karakaya A (2013) Evaluation of non-radioactive endpoints of *ex vivo* local lymph node assay-BrdU to investigate select contact sensitizers. J. Immunotoxicology 10: 1-8.

Ullmann (2005) Formaldehyde. Authors: Reuss G, Disteldorf W, Gamer AO, Hilt A. In: Ullmann's Encyclopedia of Industrial Chemistry, Wiley, VCH Verlag GmbH & Co. KGaA, pp. 1-34.

Uotila L, Koivusalo M (1997) Expression of formaldehyde dehydrogenase and *S*-formylglutathione hydrolase activities in different rat tissues. Adv. Exp. Med. Biol. 414: 365-371.

Uter W, Aberer W, Armario-Hita JC, Fernandez-Vozmediano JM, Ayala F, Balato A, Bauer A, Ballmer-Weber B, Beliauskiene A, Fortina AB, Bircher A, Brasch J, Chowdhury MMU, Coenraads PJ, Schuttelaar ML, Cooper S, Czarnecka-Operacz M, Zmudzinska M, Elsner P, English JSC, Frosch PJ, Fuchs T, Garcıa-Gavın J, Fernandez-Redondo V, Gawkrodger DJ, Gimenez-Arnau A, Green CM, Horne HL, Johansen JD, Jolanki R, Pesonen M, King CM, Krecisz B, Chomiczewska D, Kiec-Swierczynska M, Larese F, Mahler V, Ormerod AD, Peserico A, Rantanen T, Rustemeyer T, Sanchez-Perez J, Sansom JE, Silvestre JF, Simon D, Spiewak R, Statham BN, Stone N, Wilkinson M, Schnuch A (2012) Current patch test results with the European baseline series and extensions to it from the 'European Surveillance System on Contact Allergy' network, 2007–2008. Contact Dermatitis 67: 9-19.

Uter W, Rämsch C, Aberer W, Ayala F, Balato A, Beliauskiene A, *et al.* (2009) The European baseline series in 10 european countries, 2005/2006 — Results of the European surveillance system on contact allergies (ESSCA). Contact Dermatitis 61: 31-38.

Vargova M, Wagnerova J, Liskova A, Jakubovsky J (1993) Subacute immunotoxicity study of formaldehyde in male rats. Drug Chem. Toxicol. 16: 255-275.

Vaughan TL, Strader C, Davis S, Daling JR (1986a) Formaldehyde and cancers of the pharynx, sinus and nasal cavity: I. Occupational exposures. Int. J. Cancer 38: 677-683.

Vaughan TL, Strader C, Davis S, Daling JR (1986b) Formaldehyde and cancers of the pharynx, sinus and nasal cavity: II. Residential exposures. Int. J. Cancer 38: 685-688.

Vaughan TL, Stewart P, Teschke K, Lynch C, Swanson G, Lyon J, Berwick M (2000) Occupational exposure to formaldehyde and wood dust and nasopharyngeal carcinoma. Occup. Environ. Med. 57: 376-384.

Viegas S, Ladeira C, Nunes C, Malta-Vacas J, Gomes M, Brito M, Mendonca P, Prista J (2010) Genotoxic effects in occupational exposure to formaldehyde: a study in anatomy and pathology laboratories and formaldehyde-resins production, J. Occup. Med. Toxicol. 5: 1-8.

Walrath J, Fraumeni J (1983) Mortality patterns among embalmers. Int. J. Cancer 31: 407-411.

Walrath J, Fraumeni J (1984) Cancer and other causes of death among embalmers. Cancer Res. 44: 4638-4641.

Wang HX, Zhou DX, Zheng L, Zhang J, Huo YW, Tian H, Han SP, Zhang J, Zhao WB (2012) Effects of paternal occupation exposure to formaldehyde on reproductive outcomes. J. Occup. Environ. Med. 54: 518-524.

Wang M, Cheng G, Balbo S, Carmella SG, Villalta PW, Hecht SS (2009a) Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and nonsmokers. Cancer Res. 69: 7170-7174.

Ward JB, Hokanson J, Smith E, Chang L, Pereira M, Whorton E, Legator M (1984) Sperm count, morphology and fluorescent body frequency in autopsy service workers exposed to formaldehyde. Mutat. Res. 130: 417-424.

Warshaw EM, Belsito DV, Taylor JS, Sasseville D, DeKoven JG, Zirwas MJ, Fransway AF, Mathias CGT, Zug KA, DeLeo VA, Fowler JF Jr, Marks JG, Pratt MD, Storrs FJ, Maibach HI (2013) North American Contact Dermatitis Group patch test results: 2009 to 2010. Dermatitis 24: 50-59.

Weil CS and Scala RA (1971) Study of intra- and interlaboratory variability in the results of the rabbit eye and skin irritation tests. Toxicol. Appl. Pharmacol. 19: 276-360.

West S, Hildesheim A, Dosemeci M (1993) Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: results from a case-control study. Int. J. Cancer 55: 722-727.

World Health Organization — WHO (1989) Environmental Health Criteria (EHC) 89, Formaldehyde, IPCS International Programme on Chemical Safety 1989. Online: http://www.inchem.org/documents/ehc/ehc/ehc89.htm

World Health Organization (2010) WHO guidelines for indoor air quality. Selected pollutants. WHO, Geneva. Online: http://www.ncbi.nlm.nih.gov/books/NBK138705

Wilkinson JD, Shaw S, Andersen KE, Brandao FM, Bruynzeel DP, Bruze M, Cmara JMG, Diepgen TL, Ducombs G, Frosch PJ, Goosens A, Lachappelle JM, Lahiti A, Menne T, Seidenari S, Tosti A, Wahlberg JE (2002) Monitoring levels of preservative sensitivity in europe: a 10-year overview (1991-2000). Contact Dermatitis 46: 207-210.

Wilmer JW, Woutersen R, Appelman L, Leeman W, Feron V (1989) Subchronic (13 week) inhalation toxicity study of formaldehyde in rats: 8-hour intermittent versus 8-hour continuous exposures. Toxicol. Lett. 47: 287-293.

Woutersen RA, Appelman L, Wilmer J, Falke H, Feron V (1987) Subchronic (13 week) inhalation toxicity study of formaldehyde in rats. J. Appl. Toxicol. 7: 43-49.

Woutersen RA, Garderen-Hoetmer AV, Bruijntjes JP, Zwart A, Feron VJ (1989) Nasal tumors in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde, J. Appl. Toxicol. 9: 39-46. In: Schulte A, Bernauer U, Madle S, Mielke H, Herbst U, Richter-Reichhelm HB, Appel KE, Gundert-Remy U (2006) Assessment of the Carcinogenicity of Formaldehyde [CAS No. 50-00-0], BfR (Bundesinstitut für Risikobewertung), Berlin, Germany.

Ye X, Ji Z, McHale CM, Ding S, Thomas R, Yang X, Zhang L (2013) Inhaled formaldehyde induces DNA-protein crosslinks and oxidative stress in bone marrow and other distant organs of exposed mice. Environ. Mol. Mutag. 54: 705-718.

Zeller J, Hogel J, Linsenmeyer R, Teller C, Speit G (2012) Investigations of potential susceptibility toward formaldehyde-induced genotoxicity. Arch. Toxicol. 86: 1465-1473.

Zeller J, Neuss S, Mueller JU, Kahner S, Holzmann K, Hagel J, Klingmann C, Bruckner T, Triebig G, Speit GA (2011) Assessment of genotoxic effects and changes in gene expression in humans exposed to formaldehyde by inhalation under controlled conditions. Mutagenesis 26: 555-561.

Zhang L, Steinmaus C, Eastmond DA, Xin XK, Smith MT (2009) Formaldehyde exposure and leukemia: a new meta-analysis and potential mechanisms. Mutat. Res. 681: 150-168.

Zhang L, Tang X, Rothman N, Vermeulen R, Ji Z, Shen M, Qiu C, Guo W, Liu S, Reiss B, Freeman LB, Ge Y, Hubbarde AE, Hua M, Blair A, Galvan N, Ruan X, Alter BP, Xinl KX,

Li S, Moore LM, Kim S, Xie Y, Hayes RB, Azuma M, Hauptmann M, Xiong J, Stewart P, Li L, Rappaportt SM, Huang H, Fraumeni JF, Smith MT, Lan Q (2010) Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. Cancer Epidem. Biomarkers Prev. 19: 80-88.

Zhao W, Peng G, Yang X (2009) DNA-protein crosslinks induced by formaldehyde and its repair process. Int. J. Environ. Pollut. 37: 299-308.

Zwart A, Woutersen RA, Wilmer JW, Spit BJ, Feron VJ (1988) Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. Toxicology 51: 87-99.