CLASSIFICATION LABELING AND PACKAGING
OF SUBSTANCES AND MIXTURES

– Response to the Annex XV dossier submission for the harmonised classification and labeling of formaldehyde

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Summary

The CLH report proposes cat. 1A for carcinogenicity although “the existence of a grouping of cases in plant 1 of the National Cancer Institute (NCI) cohort raises a doubt on potential cofounder and lowers the level of evidence” and therefore further arguments are given as supportive evidence. But formaldehyde (FA) has a very strong epidemiology database (in total about 50,000 workers in 3 large cohort studies) and therefore the decision regarding cat. 1A should solely be based on the epidemiology data. The supportive arguments are either discussed in the sections related to cat. 1A and cat. 1B classification or in the introduction. Overall, we conclude that the former classification (cat. 2, CLP or cat. 3 DSD) should be maintained.

Apart from a discussion on carcinogenicity classification, two further sections deal with classification for mutagenicity and route specific classification.

Classification for carcinogenicity cat. 1A: we present data showing that

- no consistent evidence can be obtained from the NCI cohort. All risk estimates are driven by plant 1 and cannot be generalized as shown by an interaction analysis.
- the grouping of cases in plant 1 by the largest number of subjects exposed here to high peaks. The number of workers with highest peak exposure was larger for all other plants, but the NPC incidence was clearly lower.
- the correlation of NPC with peak exposure is rather speculative. A sensitivity analysis showed that the low p-value of 0.02 was possibly distorted downward by the small sample size (only 10 NPCs). Further

many NPCs might be related to exposures prior to entering plant 1. And finally the Hauptmann study is incomplete because of 1000 “missing deaths”. This can only be clarified by the still missing NCI update.

- the case control studies can hardly be used as supportive evidence as demonstrated by a recent metaanalysis.

Altogether, the data do not support a causal relationship between formaldehyde exposure and induction of NPC and do not correspond to a sufficient evidence of carcinogenicity in humans as required for a cat. 1A classification. The most relevant cohort study is not reliable and its update might lead to a relevant reevaluation of the relationship between formaldehyde exposure and NPC.

Classification for carcinogenicity cat. 1B: According to section 3.6.2.2.5. there are several factors that “can be viewed as either increasing or decreasing the level of concern for human carcinogenicity.”

In section 3.6.2.2.6. these factors are listed. The criteria a-f and h,i do not provide arguments for a carcinogenicity cat.2 classification and the most important criteria to differentiate between cat. 1A and 2 for FA are:

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g. structural similarity to a substance(s) for which there is good evidence for carcinogenicity

j. the possibility of a confounding effect of excessive toxicity at test doses

k. mode of action and its relevance to humans, such as *cytotoxicity with growth stimulation*, mitogenesis, immunosuppression, mutagenicity.

FA leads to cytotoxic irritation with increased regenerative cell replication in the nose of exposed rats. A threshold has been demonstrated for the increase of cell replication at =/>2ppm in rats. This has to be seen in the context of efficient

metabolic detoxification of FA in all tissues (biological half life of about 1 min); half saturation of the detoxification pathway was estimated to occur at exposure concentrations of 2.6 ppm.

Histopathological lesions are already induced in the nose of rats after a single day of exposure to the carcinogenic concentrations of 10 and 15 ppm. Extensive ulceration is already found after 4 days and squamous metaplasia after 9 days of exposure. Mild effects occur after a few days at 6 ppm. By prolonged exposure such lesions progress to hyperplasia and squamous metaplasia and finally to carcinomas.

There is a clear difference in the sensitivity of different species with regard to the carcinogenic activity of FA: mice are much less sensitive than rats and hamsters did not develop respiratory tumors after inhalation exposure.

There is no reliable experimental or epidemiological evidence or a mechanistic basis for induction of systemic tumors.

These data lead to the conclusion that tumor development depends on excessive cytotoxicity leading to increased cell proliferation. Genotoxicity is of minor importance. Without such pronounced cytotoxicity and regenerative cell proliferation no tumors will develop. In addition, a carcinogenic effect of FA is only to be expected by inhalation exposure. The toxicological profiles of FA and acetaldehyde are basically identical, apart from their potency, justifying the same classification for FA as that for acetaldehyde, namely cat. 2 under the CLP regulation.

In conclusion, apart from differences in species sensitivity, especially three of the criteria mentioned above need to be taken into consideration for a classification decision of FA leading to cat.2, namely

g. structural similarity to a substance(s) for which there is good evidence for carcinogenicity

j. the possibility of a confounding effect of excessive toxicity at test doses

k. mode of action and its relevance to humans, such as *cytotoxicity with growth stimulation*, mitogenesis, immunosuppression, mutagenicity.

In humans tumors of the upper respiratory tract may only develop under conditions of high cytotoxicity with prolonged growth stimulation. Under these considerations FA was formerly classified as a category 3 carcinogen. Since that time no additional data have been reported that might call the former classification into question. Therefore, these arguments also pertain to the new CLP regulation leading to category 2.

There are positive and negative studies for micronuclei induction in buccal or nasal cells of humans exposed to FA. These data are taken as supportive evidence in the CLH report for the classification of FA as carcinogenic cat 1A. A weight of evidence assessment showed that the negative studies of Speit et al. (2007a) and Zeller et al. (2011) carried out under strictly defined conditions are the most reliable ones to assess local mutagenicity in the upper respiratory tract of humans. The positive studies on MN induction in workers reported by other authors can by no means be taken as sufficient evidence for such a local mutagenicity. Therefore the overall database on MN induction in nasal or buccal cells in humans cannot be used as supportive evidence for a Cat.1 carcinogenicity classification. In addition the negative studies in humans are supported by an inhalation study in rats not leading to MN formation in the nose at exposures up to 15 ppm.

Classification for mutagenicity cat. 2: Classification for the different mutagenicity categories always refer to germ cell mutagenicity. Due to the high reactivity of FA, DPX, DNA adducts and DNA-DNA cross links have only been observed in the nasal tissue of rats after inhalation. Furthermore, inhalation of FA does not lead to an increase of its blood concentration. Therefore after exposure by the inhalation, oral

or dermal route, FA will not reach the germ cells and a classification for (germ cell) mutagenicity is not warranted.

In the CLH report two studies are specifically mentioned as giving support to a mutagenicity cat. 2 classification:

Dallas et al. (1992) claimed that chromosomal aberrations in lung lavage cells are induced after inhalation exposure. But this finding could not be reproduced in a recent inhalation study in which neither MN nor DNA strand breaks, alkali-labile sites or DPX were induced in lung lavage cells. This study carried out according to today's standards must be given precedence over the Dallas study. In addition, inhalation studies and mathematical modeling have shown the FA by inhalation will not reach the lung.

Migliore et al. (1989) reported MN formation in the gastric mucosa after oral application at a highly cytotoxic dose level leading to hyperemia and hemorrhage. This study can by no means be compared to the inhalation exposure situation.

In conclusion there is no reason to classify FA as a cat. 2 mutagen.

Route specific classification: It is proposed that a classification of FA should be limited to the inhalation route. A carcinogenicity classification of FA after oral exposure is not warranted for the following reasons:

- No tumors were observed in a guideline 2-year carcinogenicity bioassay
- Indications for the development of forestomach papillomas in another study are uncertain due to the unclear histopathological criteria applied
- No indications for tumor development were obtained after oral application of two other highly reactive aldehydes.

Similarly a classification for the dermal route is not warranted: In experiments with skin application FA did not act as initiator or promotor. Although treatments with FA alone were included in these experiment, a definite answer as to whether FA may be a complete skin carcinogen is not possible by these data. But as FA will only act on cells of its primary contact and taking into account the multilayered structure of the skin, a local carcinogenic activity can be excluded.

Introduction / general remarks

The CLH report proposes cat. 1A for carcinogenicity based on the arguments given on p. 11. It is accepted that “the existence of a grouping of cases in plant 1 of the National Cancer Institute (NCI) cohort raises a doubt on potential cofounder and lowers the level of evidence” and therefore further arguments are given as supportive evidence in a weight of evidence approach for the proposed classification of cat 1A.

Formaldehyde (FA) has a very strong epidemiology database (in total about 50000 workers in 3 large cohort studies) and therefore the decision regarding cat. 1A should solely be based on the epidemiology data. In this respect we refer to paragraph 1.1.1.1 in Annex I (of regulation 1272/2008) stating “where the criteria cannot be applied directly to available identified information, … the weight of evidence determination using expert judgment shall be applied“. But the broad epidemiology database can directly be applied to the decision for carcinogenicity based on human data without referring to additional information derived from other studies in a weight of evidence approach. To our opinion the epidemiological evidence does not justify a decision for cat 1A (see section of our comments on cat. 1A).

On the further supportive arguments given on p. 11 we have the following comments:

“But the grouping of cases (but) it can also be explained by the largest number of subjects exposed to high peaks in this specific plant.” This point will be addressed in the section concerning cat. 1A classification.

“Induction of tumours in the nasal cavity in rats with a proposed mode of action based on chronic irritation of the respiratory tract and local genotoxicity at doses inducing an increased proliferation.” We agree that the animal data show induction of nasal tumors after inhalation. The consequences of these experimental findings for classification are addressed in the section discussing cat. 1B vs cat. 2 and we propose cat. 2.

“Indication of local genotoxicity in exposed humans as evidenced by increases in micronuclei frequency in buccal and nasal mucosa cells in several studies.” The reliability of these data from exposed workers in comparison to studies not showing such effects are addressed in the section discussing cat. 1B vs cat. 2. The positive data are not at all conclusive and cannot be used as supportive arguments for carcinogenicity classification, nor do they warrant a classification for mutagenicity.

“Human sensitivity to FA-induced irritation, with irritation of the eye and of the nose/throat being consistently reported after exposure to formaldehyde.” This argument cannot be used for classification. All studies relating to irritation in humans only refer to sensory irritation. There is no doubt that FA is a sensory irritant but there is no reliable evidence that FA exposure has also led to cytotoxic irritation in humans. And only cytotoxic irritation with regenerative cell proliferation (but not sensory irritation per se) is a necessary prerequisite for tumor induction.

“No species-specific mechanism is evident and human data denote human sensitivity to FA effects (genotoxicity and irritation). The mode of action of carcinogenicity in the rat nasal cavity is therefore considered relevant to humans.” We agree that the mode of action for tumor induction by FA inhalation is not species dependent. If the epidemiological database had clearly shown tumors of the upper respiratory tract in humans, this effect would have been caused by the same

mechanism operative in rats. Even if the induction of NPC is plausible, it has not been proven by the epidemiology data in a sufficiently robust manner to justify cat. 1A as shown in the section discussing cat. 1A.

Our comments on the classification proposed in the CLH report are organized as follows:

In the section referring to cat. 1A we present our arguments that the epidemiological data do not warrant cat. 1A. As said above, the epidemiological database is so broad that an assessment of cat. 1A should be done on its own merits.

In the section referring to cat. 1B vs cat.2 we present our arguments why the animal data do not warrant cat. 1B, but rather support cat. 2 that corresponds to the original cat.3 classification under DSD. Here it has to be taken into consideration that basically the classification criteria are the same for the old DSD and the new CLP regulation and that the animal and mechanistic data have not changed substantially after the former cat. 3 decision.

In the following section our arguments are given why a classification for mutagenicity is not appropriate.

The final section deals with route specific classification. In this respect we propose that any classification, regardless whatever would be the final decision, should be restricted to the inhalation route.

Classification for carcinogenicity Cat 1A?

Introduction

We agree with the CLH report that any considerations for classification of FA as a human carcinogen (cat. 1A) should predominantly be based on tumors observed in the upper respiratory tract, i.e. nasopharyngeal cancer (NPC). This site corresponds to findings in experimental cancer bioassays as well as to the high reactivity of FA leading to effects only at the site of first contact like DNA protein cross links (DPX), DNA adducts or increased cell proliferation. On the other hand epidemiological data pointing to induction of leukemia are not supported by animal or mechanistic data. As this tumor type is not taken forward to justify cat. 1A, the comments presented here only refer to NPC.

We further agree that the study of Hauptmann et al. (2004) is the most important one for the evaluation of NPC and it was also pivotal for the IARC Cat. 1 decision for NPC. But this is the only cohort study reporting an increased incidence of NPC, while it is not supported by two further large cohort studies (Coggon et al., 2003; Pinkerton et al., 2004). Note that this view is supported by a couple of independent reviews (Chang and Adami 2006, Bosetti et al. 2008, Duhayon 2008; Bachand et al. 2010).

CLH summary on epidemiology (NPC)

The CLH report for formaldehyde (Version of 28 September 2011) summarized the scientific justification for the CLH proposal “Carc 1A” as follows (Section 2.2, p. 11):

“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. It is considered that the doubt of a potential cofounder is raised by the grouping of cases in the plant 1 of the NCI cohort. But considering the overall database and more specifically the fact that the grouping of cases in plant 1 can also be explained by the largest

number of subjects exposed to high peaks in this specific plant, correlation of NPC with the level of peak exposure to formaldehyde, the evidence provided by case-control studies and the biological plausibility, the doubt that the observed induction of NPC may be due to confounder can be ruled out *with reasonable confidence*.

Altogether, the data support a causal relationship between formaldehyde exposure and induction of NPC and corresponds to a sufficient evidence of carcinogenicity in humans."

Comment on the CLH summary on epidemiology

We do not agree to the following statements cited from the above passage for the reasons given below.

1) "consistent epidemiological evidence obtained from the NCI cohort".

It is necessary to take into consideration that the evidence obtained from the National Cancer Institute (NCI) cohort, i.e., Hauptmann et al. 2004, is *inconsistent*. Marsh et al. (2007b) showed by an interaction analysis - an important statistical analysis not performed by Hauptmann et al. (2004) - that the risk estimates are modified by plant. Thus, the results of this study are proven to be *inconsistent* and cannot be generalized across plants.

2) "the grouping of cases in plant 1 can also be explained by the largest number of subjects exposed to high peaks in this specific plant".

Again this statement does not reflect the total database. According to Table 2 in Marsh and Youk (2005) the following description is correct: Plant 1 comprised the

highest number of workers with highest peak exposures (n=1964) leading to 6 NPC cases among the exposed. In plants 2-10 the number of workers with highest peak exposures was clearly greater (n=4293) but only 2 NPC cases were observed among the exposed and 2 NPC cases were observed in the unexposed as defined by Hauptmann. Note further, that the NPC risk at Plant 1 is significantly different from all other plants even after taking cumulative and peak exposures into account, i.e., the elevated NPC risk cannot be explained by higher exposures in Plant 1 (Marsh et al. 2007b).

3) "can also be explained by the … correlation of NPC with the level of peak exposure to formaldehyde"

There are several reasons why this statement cannot be taken as proven but rather is speculative.

First, as the evidence for the association between NPC and Formaldehyde is only based on 10 cases in the Hauptmann study, Marsh et al. (2007b) carried out a sensitivity analysis to look for indications of a small sample bias that often tends to exaggerate risk estimates and to produce artificially low p-values. They performed a systematic sensitivity analysis by adding repeatedly one additional NPC case to all of the 117 different exposure situations. Only 42% of the scenarios returned a p-value < 0.05. Thus, the p-value of 0.02 belonging to the Hauptmann analysis was not representative and possibly was distorted downward by a small sample bias. This analysis showed that the "correlation" was much more unstable than the p-value reported by Hauptmann et al. may make believe.

Second, an explorative study by Marsh et al (2007a) indicated that 5 out of 7 NPC cases from plant 1 (only 6 cases according to Hauptmann et al. 2004) might be

associated with exposure to silver smithing or other metal work before they were hired at the plant. This also sheds doubt on the “correlation”.

Third, the Hauptmann et al. (2004) study is incomplete. The authors failed to perform a sufficiently complete follow-up in mortality. They documented 8486 deaths but missed approximately 1000 deceased. These “missing deaths” were not evenly distributed, but the percent increase in revised deaths among “unexposed” workers was twice that of the “exposed” for all deaths, all cancer deaths, and all solid neoplasms. If these missing deaths are taken into account, there is a decrease in the relative risks in comparison to the original calculations, e.g. for all lymphohematopoetic malignancies or leukemia. NCI, however, did not report on the effect of these missing deaths on NPCs. Although Marsh et al. (2010) highlighted the problem of these missing deaths for the analysis of NPC no further information has been published in this regard by NCI.

4) “can also be explained by … the evidence provided by case-control studies “

The case control studies have to be assessed in a more detailed manner. Bachand et al. (2010) performed a meta-analysis and found significantly increased odds ratios in case-control studies, but the summary odds ratio for smoking adjusted studies was no longer significantly increased with an unexceptionable estimate of 1.10 (95% CI: 0.80, 1.50). Thus, the above statement about case-control studies is invalid when smoking habits are taken into account.

Altogether, the data do not support a causal relationship between formaldehyde exposure and induction of NPC and do not correspond to a

sufficient evidence of carcinogenicity in humans as required for a cat. 1A classification. In addition it has to be taken into account that the database of the most relevant cohort study is not reliable and that the soon to be expected NCI cohort study update might lead to a relevant reevaluation of the relationship between formaldehyde exposure and NPC.

Classification for carcinogenicity cat. 1B?

Regulatory situation

Section 3.6. of Regulation (EC) No 1292/2008 (16 December 2008) relates to carcinogenicity.

According to section 3.6.2.2.5. there are several factors that "can be viewed as either increasing or decreasing the level of concern for human carcinogenicity."

These are listed in section 3.6.2.2.6. as follows:

a. "tumor type and background incidence
b. multi-site responses
c. progression of lesions to malignancy
d. reduced tumor latency
e. whether responses are in single or both sexes
f. whether responses are in a single species or several species
g. structural similarity to a substance(s) for which there is good evidence for carcinogenicity
h. routes of exposure
i. comparison of absorption, distribution, metabolism and excretion between test animals and humans
j. the possibility of a confounding effect of excessive toxicity at test doses

k. mode of action and its relevance to humans, such as *cytotoxicity with growth stimulation (emphasis added)*, mitogenesis, immunosuppression, mutagenicity."

Assessment of the criteria a-k given in section 3.6.2.2.6

a) tumor type and background incidence

Inhalation of FA at high concentrations leads to the induction of nasal tumors starting around 6 ppm (Kerns et al., 1983; Monticello et al., 1996). The dose response curve is highly non-linear. The tumors originate from the respiratory epithelium in the anterior part of the nose (Morgan et al., 1986a). The spontaneous incidence of squamous cell carcinomas in the nose is very low (Conolly et al., 2003).

b) multi-site responses

FA inhalation has only resulted in nasal tumors in experimental animals.

c) progression of lesions to malignancy

Already after 1 day of exposure to the clearly carcinogenic concentrations of 10 and 15 ppm epithelial cell degeneration, single cell necrosis and epithelial exfoliation were observed. These lesions progressed to local ulceration, epithelial hyperplasia and squamous metaplasia after 4-9 days of exposure. Lesions at 6 ppm were only mild single cell necrosis and patchy hyperplasia (Swenberg et al., 1983a; Morgan et al., 1986b, Monticello et al., 1991). Exposure over 6 weeks and longer led to clear epithelial hyperplasia and squamous metaplasia at 10 and 15 ppm and mild effects at 6 ppm. No lesions were found at 2 ppm and below (Monticello et al., 1996). Thus, at carcinogenic exposure concentrations lesions progressed with exposure duration from single cell necrosis and local ulceration via epithelial hyperplasia and squamous metaplasia finally to squamous cell carcinomas.

d) reduced tumor latency

Exposure to high FA concentration led to a clear reduction of tumor latency. First tumors already became apparent within the first year of exposure to 15 ppm (Swenberg et al., 1980; Albert at al., 1982; Sellakumar et al., 1985).

e) whether responses are in single or both sexes

The carcinogenic response in the nose of rats is independent of the sex (Kerns et al., 1983).

f) whether responses are in a single species or several species

Mice (Kerns et al., 1983) and hamsters (Dalbey, 1982) are by far less susceptible to nasal tumor induction after inhalation exposure to FA than rats. There is no indication for such an effect in hamsters, but in mice at very high concentrations these tumors may occur as evidenced by 2 nasal tumors at 15 ppm.

g) structural similarity to a substance(s) for which there is good evidence for carcinogenicity

As FA is by far the best investigated chemical within the group of aldehydes, data derived from other aldehydes do not add much further evidence to evaluate the carcinogenic potential of FA. Aldehydes with a carcinogenic or mutagenic effect have been evaluated by the German MAK Commission and the evaluations for acetaldehyde (MAK, 2008), acrolein (MAK, 1997) and glutaraldehyde (MAK, 2002, 2006) are briefly summarized here. All these substances have been tested for carcinogenicity, either by oral or inhalation exposure. They all exhibit a genotoxic potential in different test system.

Acetaldehyde (MAK, 2008): similar to FA, acetaldehyde is an endogenous metabolite and endogenous DNA adducts have been found. After inhalation

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exposure the olfactory epithelium is more susceptible to acetaldehyde than the respiratory epithelium. Already 5 weeks of exposure to 243 ppm lead to degeneration of the olfactory and 3 days at 750 ppm to single cell necrosis. After 26 weeks at 1500 ppm hyper- and metaplasia of the respiratory epithelium are observed. Long term exposure leads to a high incidence of adenocarcinoma of the olfactory epithelium at 750 ppm, while squamous cell carcinoma of the respiratory epithelium only occurred at 1500 ppm. It is assumed that similar to FA local tissue damage is a prerequisite for tumor induction. But due to the lack of detailed dose response data a final decision is not possible whether the carcinogenic response is primarily caused by a genotoxic or a cytotoxic mechanism.

Acrolein (MAK, 1997): this substance with a double bond in conjugation to the carbonyl group is highly reactive to nucleophilic substances. This explains its severe local irritation and genotoxicity (among others leading to DNA adducts in vivo) similar to FA. In vivo mutagenic or cytogenetic effects have not been found. Increased cell proliferation and slight histopathological changes in the nasal epithelium of rats were already observed after inhalation exposure for 3 days at 0.25 ppm. In a 90 day study rats developed histopathological alterations (e.g. hyper- and metaplasia) in nasal tissue at 0.4 ppm and a NOAEL could not be established. In comparison with FA, the histopathological lesions at 0.67 ppm after 3 days of inhalation exposure corresponded approximately to those observed with FA at 3.2 ppm. A further comparison with FA is not possible since a carcinogenicity study by inhalation is not available for acrolein. The results of an oral carcinogenicity study are given in the section on exposure routes.

Glutaraldehyde (MAK, 2002, 2006): for genotoxicity/mutagenicity positive and negative results have been reported in in vitro systems, while in vivo after oral and inhalation exposure mutagenic effects were not found. In a 13 week study with exposure levels between 0.0625 and 1 ppm increased cell proliferation of the

squamous cell epithelium in the nasal vestibulum occurred in rats at 0.25 ppm and in mice already at 0.0625 ppm. Persistent metaplasia of the respiratory epithelium was found in both species starting at 0.5 ppm. Thus, in contrast to FA leading predominantly to effects in the respiratory epithelium, for glutaraldehyde the vestibulum is the most sensitive part of the nose. A 2-year carcinogenicity study has been carried out with rats (0.25, 0.5, 0.75 ppm) and mice (0.0625, 0.125, 0.25 ppm). In rats hyperplasia and inflammation of the squamous epithelium started at 0.25 ppm in the nasal vestibulum and at 0.5 ppm in the respiratory epithelium. In mice already at 0.0125 ppm metaplasia of the squamous epithelium was observed. In comparison to FA inflammation predominated in the anterior parts of the nose. Neither local nor systemic tumors were observed. A dosimetric comparison for the induction of hyperplasia and squamous cell metaplasia showed that 0.5 and 0.75 ppm glutaraldehyde would correspond to about 6 and 10 ppm FA. But at these comparable concentrations glutaraldehyde did not lead to preneoplastic changes in contrast to FA. The lack of a local carcinogenic activity may either be explained by the relationship of genotoxicity vs cytotoxicity or by the predominant action on the anterior nose covered by the more resistant squamous epithelium. The results of oral carcinogenicity studies are given in the section on exposure routes.

With the framework of EU regulations acetaldehyde has been classified as carcinogenic cat. 3 (under the old DSD regulation), but not acrolein and glutaraldehyde due to lack of positive carcinogenicity data. As classification is hazard based, the similarities in the toxicological profile between GA and acetaldehyde, apart from potency, justify FA to be treated like acetaldehyde.

In summary, there are some other aldehydes that can be assessed in parallel to FA, namely acetaldehyde, acrolein and glutaraldehyde. All of these aldehydes are genotoxic and for acetaldehyde, similar to FA, endogenous DNA adducts have been found. Acetaldehyde is carcinogenic after inhalation but due to its lower reactivity at

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much higher concentrations. The biological activities of acrolein (with a double bond in conjugation to the aldehyde function) and glutaraldehyde (with two carbonyl groups) are much higher than that of FA. For acrolein no carcinogenicity study by inhalation is available and glutaraldehyde did not lead to tumors at clearly cytotoxic concentrations. This may either be explained by the relationship of genotoxicity vs cytotoxicity or by the predominant action on the anterior nose covered by the more resistant squamous epithelium.

The similar toxicological profiles of acetaldehyde and FA justify the same classification of FA as for acetaldehyde, i.e. cat 3 (old DSD system).

h) routes of exposure

A clear carcinogenic response was only observed in rats after inhalation exposure. Findings after oral and dermal exposure will be discussed in more detail in the section on exposure route.

i) comparison of absorption, distribution, metabolism and excretion between test animals and humans

Metabolism: Glutathione-dependent cytosolic FA dehydrogenase (FDH) is the most efficient detoxifying enzyme system (Uotila and Koivusalo, 1989). FDH is highly conserved in all species (Jörnvall et al., 2000). It was found in all tissues investigated (Julia et al., 1987; Uotila and Koivusalo, 1996; Haselbeck and Duester, 1997), including the respiratory tract and nasal respiratory and olfactory mucosa (Keller et al., 1990; Casanova-Schmitz et al., 1984a; Maier et al., 1999). Metabolic detoxification leads to a rapid metabolism of FA with a biological half life of 1-1.5 min (Rietbrock, 1965, 1969; Malorny et al., 1965; McMartin et al., 1979). The detoxification pathway via formaldehyde dehydrogenase is half saturated in rats at exposure concentrations of 2.6 ppm (Casanova et al., 1989).

Endogenous vs. exogenous FA: The total endogenous FA production in humans has been calculated to be 2450 mg/h (Cascieri and Clary, 1992) and the amount detoxified in the liver as 1320 mg/h (Owen et al., 1990). Endogenous FA concentrations (free and reversibly bound) in blood and tissues of humans and animals are in the range of a few µg/g wet tissue weight (Heck et al., 1982, 1985; Casanova et al., 1988).

Inhalation of 6 or 14.4 ppm (rats) (Heck et al., 1982, 1985), 6 ppm (monkeys) (Casanova et al., 1988), or 1.9 ppm (humans) (Heck et al., 1985) did not lead to an increase of the FA concentrations in blood. In some of these studies the time span between end of exposure and analysis might have been too long allowing for metabolic degradation of exogenous FA by taking into account the short biological half life of FA. Therefore a new study was carried out. Rats were exposed to 10 ppm 13C-FA over 6 h. Blood was withdrawn during exposure (at 3 h), directly after exposure and at some time points thereafter. The sensitivity of the method allowed to determine exogenous labelled FA in the blood at a concentration of about 1.5% of that of endogenous FA. No increase of FA stemming from the exogenous labelled substance could be detected in the blood at any time point (Kleinnijenhuis, Staal, 2011). A mathematical model for the absorption and metabolism of FA vapor showed that FA is rapidly removed by the nasal tissue and the increase of FA in blood was insignificant compared to preexisting blood concentrations (Franks, 2005).

Species differences: FA is a nasal irritant leading to reflectoric depression of respiratory rate and minute volume in rats and mice. This response is much more pronounced in mice as compared to rats (Chang et al., 1981, 1983; Jaeger and Gearhart, 1982) leading to a markedly reduced delivered dose at the nasal surface in mice in comparison to rats. The difference in delivered dose is a good semi-quantitative explanation for the different responses of rats and mice to nasal tumor induction (Barrow et al., 1980, 1986).

Deposition sites identified by cell proliferation and DNA- protein cross links (DPX): Histopathological lesions and cell proliferation rates in the nasal passages of rats correspond well to the sites of tumor development after exposure to FA (Monticello et al., 1991, 1996; Casanova et al., 1994). In the monkey at 6 ppm the lesions and increased cell proliferation are not confined to the nose but extend to the larynx, trachea and carina but in much smaller quantities (Monticello et al., 1989; Heck et al., 1989). No effects were found in the maxillary sinus of monkeys (Heck et al., 1989; Casanova et al., 1994) and for DPX formation in the proximal lung and bone marrow. In rats DPX formation only occurs in the nasal respiratory mucosa with a good correlation to the sites for tumor development, but not in the olfactory mucosa or bone marrow.

j) the possibility of a confounding effect of excessive toxicity at test doses

Alterations of the nasal epithelium already occur after a single exposure to the carcinogenic concentration of 10 and 15 ppm with progression to extensive ulceration after 4 days and hyperplasia and squamous metaplasia after 9 days of exposure. Early squamous metaplasia was already detected after 4-5 days at 15 ppm. Much less severe lesions are induced by 6 ppm with only minimal focal hyperplasia and squamous metaplasia starting after exposure durations of 9 days (Swenberg et al., 1983a; Morgan et al., 1986b; Monticello et al., 1991). No histopathological abnormalities were observed at 0.7 and 2 ppm for exposures up to 18 months and only mild alterations at 6 ppm (Swenberg et al., 1986). Severe histopathological lesions were noted in the nose of rats after prolonged exposure to the carcinogenic concentrations of 10 and 15 ppm (Monticello et al., 1996; Casanova et al., 1994).

Initial increases of cell proliferation that were noted in an early investigation after exposure to 0.5 and 2 ppm returned to control rate after 3-9 days; increased cell

proliferation at 6 ppm observed after 6 weeks returned to base line after 3 months (Monticello et al., 1991, 1996).

Thus, excessive toxicity already after a few days of exposure at carcinogenic exposure concentrations leading to an increase in cell proliferation has been described in many experiments and is obviously a prerequisite for tumor development. At low exposure concentrations up to 6 ppm initially increased cell proliferation decreased and eventually returned to control levels with prolonged exposure.

The guidance for classification according to CLP defines this criterion j among others as follows (p. 204): “Excessive toxicity, for instance toxicity at doses exceeding the MTD, can affect the carcinogenic responses in bioassays. Such toxicity can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which can lead to tumour development as a secondary consequence unrelated to the intrinsic potential of the substance itself to cause tumours at lower less toxic doses.” And also the CLH report states in this respect (p.11): “Data investigating the mode of action support the existence of a threshold type mode of action for its carcinogenic properties based on the cytotoxic effect of formaldehyde. Genotoxicity is also expected to play a role above this threshold.”

Thus the criterion j has to be taken into consideration for the carcinogenicity classification of FA.

k) mode of action and its relevance to humans, such as *cytotoxicity with growth stimulation*, mitogenesis, immunosuppression, mutagenicity

The underlying mode of action for the carcinogenic effect of FA in rats after inhalation is regenerative cell proliferation caused by cytotoxicity. Various genotoxic and mutagenic effects of FA have been described in vitro, but there is no reliable evidence for mutagenicity in vivo (see section on mutagenicity). Therefore,

genotoxicity is considered to be of minor importance for tumor development. No mutations were detected in the p53 and K-ras genes in rats after inhalation exposure (Meng et al., 2010). Tumor development, cytotoxicity, cell proliferation and formation of DNA-adducts and DPX all show a highly non-linear dose response relationship that has also been demonstrated for gene expression (Andersen et al., 2008). Without cytotoxic irritation tumor development in the respiratory tract is not to be expected (MAK, 2000; McGregor et al., 2006).

The guidance for classification according to CLP defines this criterion k among others as follows (p. 205): “the existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g., hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation) may lead to a downgrading of a Category 1 to Category 2 classification.” Again this criterion is fulfilled for the local action of FA.

For systemic tumors caused by FA exposure there is no convincing evidence from animal experiments. Furthermore, mechanistic studies have not given any plausible mechanism how such tumors at distant sites from the port of entry might develop (Heck and Casanova, 2004). Mechanisms recently proposed for leukemia induction (Zhang et al., 2009, 2010) are far from conclusive. In contrast, there is strong mechanistic evidence that a genotoxic activity of FA in the form of DNA adducts is only restricted to the site of first contact (Lu et al., 2010, 2011; Moeller et al., 2011).

Mutagenicity: it is recognized that genetic events are central in the overall process of cancer development

Genotoxicity/mutagenicity is an important criterion to be taken into account for carcinogenicity classification. Therefore the basic data are summarized here. Special emphasis is given to the interpretation of mutagenic effects observed at the site of direct contact, especially in nasal and buccal cells of humans. Such findings would

be important for the interpretation of the induction of tumors in the upper respiratory tract.The classification for mutagenicity per se is addressed in a separate chapter.

Gentoxicity/mutagenicity in vitro: FA induced gene mutations in bacteria but these effects cannot be directly translated to mammalian systems because bacteria are lacking histones and therefore the predominant genotoxic effect in mammalian cells, i.e. DPX formation, is not possible in the same manner. Gene mutations in mammalian cells are of minor importance, and FA primarily leads to clastogenic effects via DPX formation generally associated with cytotoxicity (Merk and Speit, 1998; Speit and Merk, 2002). Furthermore, there is an indication for a practical threshold for induction of MN in vitro (Speit et al., 2007).

As regards genotoxicity, the standard alkaline comet assay generally is negative (Speit et al., 2007). Similar to MN induction, in vitro studies showed that SCE induction is associated with cytotoxicity and there is an indication for a practical threshold under in vitro conditions (Speit et al., 2007). SCE inducing DNA lesions are rapidly repaired (Neuss and Speit, 2008). DPX are rapidly repaired in various cell lines (Cosma and Marchok, 1988; Schmid and Speit, 2007; Speit et al., 2007, 2008). Co-cultivation experiments with the endpoints of SCE (Neuss and Speit, 2008) and DPX (Neuss et al., 2010) showed that FA after having entered a cell is not passed on to neighbor cells.

Systemic genotoxicity/mutagenicity in vivo: such systemic effects might be related to the induction of systemic tumors like leukemia. But as leukemia is not a criterion taken forward in the CLH dossier for carcinogenicity classification, reports on systemic genotoxicity/mutagenicity are only briefly summarized here. In vivo animal studies did not show systemic genotoxic (SCE, DNA strand breaks, DPX) or mutagenic effects (MN, chromosomal aberrations) after oral or inhalation exposure

(Jensen et al., 1982; Natarajan et al., 1983; Kligerman et al., 1984; Dallas et al., 1992; Morita et al., 1997; Speit et al., 2009). Inhalation exposure to exogenous [13CD2]FA did not lead to an increase of DNA adducts and DNA-DNA cross links in all tissues investigated far off the site of first FA contact. But in all of these tissues such DNA modifications caused by endogenous FA were observed (Lu et al., 2010). Similarly, no DNA adducts caused by exogenous FA were found in the bone marrow of exposed monkeys, but adducts cause by endogenous FA were clearly identified (Moeller et al., 2011).

Systemic genotoxic and mutagenic effects are reported for exposed humans in some studies (with other studies being negative). By a weight of evidence evaluation and taking account of mechanistic information these data do not allow the conclusion that FA leads to systemic (Heck and Casanova, 2004; BfR, 2006; Speit et al., 2009) genotoxic or mutagenic effects in exposed humans.

Local genotoxicity in vivo (experimental animals): genotoxic effects caused by FA at the site of first contact are known since decades and have recently been confirmed by a highly sensitive MS method. DPX have been demonstrated in nasal tissue at the predilection sites for tumor formation after inhalation exposure in rats (Casanova et al., 1989) and in addition in monkeys (Casanova et al., 1991) with a non-linear dose response relationship. DPX are rapidly repaired in vivo (Casanova et al., 1994). Point mutations of the p53 gene were identified in nasal carcinomas (Recio et al., 1992) but it was later demonstrated that FA per se does not induce such mutations in the p53 or K-ras gene (Meng et al., 2010). Inhalation exposure to exogenous [13CD2]FA led to an increase of DNA adducts and DNA-DNA cross links in the nasal epithelium in rats(Lu et al., 2010, 2011) and to DNA adducts in monkeys (Moeller et al., 2011). A highly non linear dose response relationship was described

for the dG DNA adduct found in rat nasal tissue after inhalation exposure to exogenous labelled FA. At exposure levels of 0.7, 2.0, 5.8, 9.1 and 15.2 ppm the dG adducts derived from exogenous FA amounted to 1, 3, 20, 60 and 260 % of those formed endogenously (Lu et al., 2011).

Local mutagenicity in the upper respiratory tract (experimental animals and humans): The question of local mutagenicity in the upper respiratory tract may be of major importance for a decision on carcinogenicity classification of FA. There are positive and negative studies for micronuclei in buccal or nasal cells of humans exposed to FA. These data are taken as supportive evidence in the CLH report for the classification of FA as carcinogenic cat 1A as stated on p. 11:

“Indication of local genotoxicity in exposed humans as evidenced by increases in micronuclei frequency in buccal and nasal mucosa cells in several studies.”

Therefore these findings will be discussed here in detail.

Inhalation exposure may affect both buccal and nasal cells, the latter even to a larger extent, as FA acts primarily on the upper respiratory tract. But studies investigating MN in workers must be carefully evaluated because many factors are difficult to control, like coexposure to other chemicals or life style of workers and control subjects. Above all, the study protocols for nasal and buccal cells are by no means yet standardized or validated. This is taken into account by an assessment of BfR (2006) and Appel et al. (2006) concluding that these studies are not sufficiently standardized, not fully or sufficiently reliable and the results are difficult to interpret. Similarly, a review of Speit, Schmid (2006) of studies specifically related to this endpoint cautioned that the data may suggest an increase in MN frequencies, but as there are methodological shortcomings and limited documentation the local

genotoxicity of FA in humans can presently not be fully assessed. Thus, two independent reviews have questioned the reliability of the database on local MN induction in workers.

First of all, the positive findings in humans are contradicted by an animal study with high, well defined exposures. In rats exposed by inhalation up to 15 ppm over 4 weeks no increase in MN frequency was found in nasal epithelial cells (Speit et al., 2011). There is no reason to assume that the nasal tissue of rats is much less sensitive to the action of FA than that of humans.

In the following the studies on micronuclei (MN) formation in exfoliated nasal and buccal cells of humans will be assessed in detail to come to a comparative weight of evidence evaluation. Thereby the following more general factor should be taken into consideration:

1. The histological structure of the epithelium: the epithelium of the buccal mucosa is about 40-50 cell layers thick, while the sublingual and respiratory mucosa have fewer cell layers (Speit, Schmid, 2006). As it has been shown in vitro that FA is not passed on from the cell of primary contact to neighbour cells (Neuss and Speit, 2008; Neuss et al., 2010) it is not very likely that FA after inhalation may reach the dividing basal cells in which MN may be induced.
2. The regeneration time of the epithelium: the time for buccal cells to emerge from the basal cell layer and exfoliate is estimated to be about 7-16 days with a peak of 8-21 days (Speit, Schmid, 2006) or 7-10 days (Titenko-Holland et al., 1996), while the estimated maximum lag time for nasal cells was estimated to be 16 days (Titenko-Holland et al., 1996). Therefore, Titenko-Holland et al. (1996) proposed to use besides a cumulative 90 day exposure estimate a cumulated dose only over the last 7-10 days before sampling as dose metric. But as the data for lag times are limited and lack consistency it is premature to give general recommendations for the optimal time point for MN analysis. Therefore in the comparative tables below different

exposure metrices are given and the cumulative exposure over 2 weeks is used as an appropriate metrix to compare the different studies with each other.

3. A sufficient number of cells should be scored; according to Titenko-Holland et al. (1996) and Speit, Schmid (2006) about 1500-2000 cells are necessary.
4. Differences in scrapings may affect the results as MN are less frequent in superficial layers of the oral mucosa (Speit, Schmid, 2006). This may especially play a role when scrapings are done at different times and particularly by different persons.
5. Background frequencies: according to Speit, Schmid (2006) the "Human Micronucleus Project" reported average MN frequencies in the normal human population of 1-3‰ with no significant variations between different types of exfoliated cells. But within the studies to be discussed, several control frequencies were clearly outside this range.

In the assessment of MN in exfoliated cells by the CLH report (p. 84) it is stated that the negative results in the two volunteer studies may be due to

- the lower exposure in particular to peaks
- and to the small number of subjects.

The following tables give an overview of the most relevant parameters of the studies investigating MN in epithelial buccal or nasal cells. Many of these studies were included in the review of Speit, Schmid (2006) but the study of Kitaeva et al. (1996) was excluded because the cells from students were scored after a primary one-time contact with FA and positive findings were obtained 24-48 h thereafter (this time span is too short considering the kinetics of MN formation).

Further comments on the studies listed in the table:

Ballarin et al. (1992): workers in a plywood factory, the exposure levels were given as 0.1 and 0.39 mg/m³ (0.08 and 0.32 ppm). Assuming that the workers worked 8

h/d, 5 d/week this would lead to a cumulative exposure over the last 2 weeks of 6.4 and 25.6 ppm x h. There were two groups of workers with a marked difference in exposure: 7 warehouse workers: mean TWA (8h) 0.32 ppm; 8 workers in sharing/pressing/sawmill: mean TWA (8h) ~0.1 ppm. While for both groups combined there was a significant difference in MN to the control group (0.90 vs 0.25 %o, $p<0.01$), there was no significant difference within these two exposure subgroups (0.32 ppm: 0.97 %o; 0.1 ppm: 0.74 %o). The close resemblance of MN frequencies in both subgroups with a clear difference in exposure level (no dose response relationship) remained unexplained. But it is to be noted that all workers were exposed to wood dust at concentrations varying between 0.11 and 0.73 mg/m³ leading to the possibility that wood dust might have been a major factor for induction of MN in both groups.

Studies of MN in buccal cells (positive studies are marked by a bold reference)

Ref**	No of cells	Stain	Location scraping	Number of		Mean MN in %o (p)		Exposure (ppm)		
				Contr	Exp.	Contr	Expo.	TWA (h)	Peak	Cumul *
No92		A O	nr	34	28	3.4	7.6	0.1, 0.2, 0.3 (different factories)	nr	8-24
Su93	1500	F/FG	Cheek	29	29	0.046	0.60 (<0.05)	1.4 (125 min);0.3 (8h) estimated, range 0.15-4.3	nr	5
Ti96	561-4113	FISH	Cheek	19	19	0.6 (t) 0.1 (-)	2.0(t) (0.007) 0.9(-) (0.005)	Similar to Su93	nr	1.2
Yi97	~3000	Wright	Cheek	25	25	0.568	0.857 (<0.01)	0.41; 3h/d, 3d/wk	1.28	7.4
Bu02	3000	F/FG	Cheek	18	28	0.33	0.71 (<0.05)	2-4, 8 h/week	nr	32-64
Sp07	2000	DAPI	Cheek	21	21	0.95; 0.86	1.33; 0.94; 0.85; 0.44 (ns)	Up to 0.5, 4 h/d	1	13.5
Vi10	2000	F	cheek	85	30(f) 50(a)	0.13	f:1.27(<0.001) a:0.64(<0.005)	f: 0.21 a: 0.28	f: 0.52 a: 2.52	f:14.7 a:19.6
La11	2000	F	Cheek?	85	56	0.81	0.96 (0.002)	0.16	1.14	11.2

*: cumulative exposure over the last 2 weeks (ppm x h). If no details are given, the exposure duration for workers is assumed to be 8 h/d, 5 d/week over 2 weeks

**: references are given as the first two letters of the first author and the last two digits of the year of publication

nr: not reported. ns: not significant (t): total MN. (-): centromere negative MN

I T: inner turbinate. If T: inferior turbinate

F/FG: Feulgen / Fast Green. A O: acridine orange FISH: FISH with centromere probe

DAPI: DAPI/propidium iodide and fluorescence microscopy. F: Feulgen without counterstain

f: workers in a FA/FA resin factory. a: workers in a anatomy/pathology laboratory


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Studies of MN in nasal cells (positive studies are marked by a bold reference)

Ref**	No of cells	Stain	Location scraping	Number of		Mean MN in %o (p)		Exposure (ppm)		
				Contr	Exp.	Contr	Expo.	TWA (h)	Peak	Cumul*
Ba92	6000	F/FG	I T	15	15	0.25	0.90 (<0.01)	0.08-0.32 (8h)	nr	6.4-25.6
Su93	1500	F/FG	If T	29	29	0.41	0.50 (0.26, ns)	1.4 (125 min);0.3 (8h) estimated, range 0.15-4.3	nr	5
Ti96	394-5770	FISH	If T	13	13	2.0 (t) 0.5 (-)	2.5(t) (0.2,ns) 1.0(-) (0.03)	Similar to Su93	nr	1.2
Yi97	~3000 ?	Wright	Septum	25	25	1.2	3.85 (<0.001)	0.41; 3h/d, 3d/wk	1.28	7.4
Bu01	3000	F/FG	I T	25~1.4	23	0.61	1.01 (<0.01)	2-4, 8 h/week	nr	32-64
Ye05	3000	Wright	septum	23	18	1.25	2.70 (<0.05)	0.8, 8 h/d, 6 d/wk	~1.4	76.8
Ze11	2000	DAPI	I T	41	41	0.21	0.27; 0.24; 0.24; 0.17 (ns)	Up to 0.7, 4 h/d	0.8	8.3

For explanations see table on buccal cells

Norppa et al. (1992): this study is reported as an abstract only. Workers in a plywood factory, in a chipboard impregnation facility and in fibre glass production were investigated. Therefore at least for part of the workforce co-exposure to wood dust is to be assumed. Calculation of cumulative exposure was carried out as described for Ballarin et al. (1992) leading to a 2 week cumulative exposure of 8-24 ppm x h. The MN frequency in the control group is very high as compared to the other studies and the proposal of the "Human Micronucleus Project". Although the exposure at the 3 factories was clearly different, the same results were obtained for each factory separately (no dose response relationship). This may indicate to an unknown confounding co-exposure.

Suruda et al. (1993): study on mortician students during embalming course; the MN frequencies pre-course served as control value in comparison to the post-course value. The cumulative exposure over the whole course was calculated as 14.8 ppm x h. If it is assumed that 1/3 of all embalmings concentrated during the last 2 weeks of the course, a cumulative 2-week exposure of 5 ppm x h would be obtained. This value is taken as the relevant cumulative exposure. Only for buccal cells (not for nasal cells) a significant pre- vs post-course increase was noted, but the control value (0.046 %o) was extremely low and only 2 students had any baseline MN, both females. Thus the increase during the embalming course is difficult to interpret.

Titenko- Holland et al. (1996): follow up study of Suruda et al. (1993); unstained slides of the Suruda study were used for FISH centromere probe to differentiate between aneugenicity and clastogenicity. MN appeared to be mainly caused by clastogenicity. 2 exposure metrices were calculated: whole course cumulative as in the Suruda study and 7-10 day cumulative with the following results:

buccal cells: whole course 14.8 ppm x h; 7-10 day: 1.2 ppm x h

nasal cells: whole course 16.5 ppm x h; 7-10 day: 1.9 ppm x h

The 7-10 day exposure is taken as relevant. Basically the results of Suruda were confirmed: the effect in buccal cells was significant for both total and centromere negative MN, while in nasal cells there was a significant post-course increase only for centromere negative MN. It should be noted that the quality of the slides might in some cases not have been sufficient, as only low numbers of cells could be scored (<1500 as recommended) for some subjects, although the average number of cells scored was in the range of the recommended 1500/subject. There was no correlation of the MN increase with both of the exposure metrices used.

Ying et al. (1997): students in an anatomy course over 8 weeks, the MN frequencies pre-course served as control value in comparison to the post-course value. Exposure 3 h/d, 3 times a week. The mean exposure of 0.41 ppm would give a cumulative exposure over the last 2 weeks of 7.4 ppm x h.

Burgaz et al. (2001): pathology and anatomy staff analyzed for nasal cells. No appropriate local ventilation and no personal protection. Exposure assessment only by stationary measurement: 2-4 ppm. Exposure duration 8 h/week for 20 subjects, for 3 subjects only 2 h/week. This leads to a 2-week cumulative exposure for the majority of the subjects of 32-64 ppm x h. As only stationary measurements are available, the actual exposure of the subjects is very uncertain.

Burgaz et al. (2002) and erratum (2006): pathology and anatomy staff analyzed for buccal cells. By comparison of the exposure condition, the subjects were obviously drawn from the same working place as those studied by Burgaz et al. (2001). In addition a group of shoemakers was investigated without exposure to FA, but to relatively high concentrations of n-hexane (mean ~58 ppm), toluene (mean ~26 ppm)

and methy ethyl ketone (mean ~11 ppm). Although these solvents are generally thought not to lead to mutagenic effects, there was a statistically significant increase in MN frequency in the exposed shoemakers as compared to controls (0.62 vs 0.33 %o). The anatomy workers were exposed to the same range of these solvents and in addition to FA. The mean MN frequency in this group (0.71 %o) was very close to that of the shoemakers and also statistically significantly different from the control group. Thus, in principle it cannot be decided whether the MN were caused by FA or the other solvents.

Ye et al. (2005): this study on highly exposed workers in FA manufacturing (~0.8 ppm) and waiters exposed to low concentrations (~0.09 ppm) was already carried out in 1992. Exposures were determined by environmental monitoring. The exposure situations were as follows:

Waiters: 5 h/d, 7 d/week leading to a 2-week cumulative exposure of 6.3 ppm x h

Workers: 8 h/d, 6 d/week leading to a 2-week cumulative exposure of 76.8 ppm x h.

While there was a statistically significant increase in MN noted for the workers in comparison to the control group, this was not found for the group of waiters.

Speit et al. (2007a): investigation on buccal cells in volunteers exposed over 2 weeks (4 h/d) at defined exposures of 0.15-0.5 ppm with peaks up to 1 ppm. Cumulative exposure over 2 weeks was 13.5 ppm x h. Cell sampling before (2x) and directly after the last exposure and 1, 2 and 3 weeks thereafter. This sampling strategy should enable to capture the optimal time point according to the cell cycle of the oral mucosa. The MN frequencies pre-exposure served as control value. The authors noted that for MN in buccal cell no clear positive control substance has been established. Upm to now clearly reproducible effects have not been found in oersons with a clearly defined exposure, like for cancer patients under chemotherapy.

Viegas et al. (2010): the authors studies 2 groups of workers: anatomy/pathology workers and workers in FA and FA-resin production. It is noted that the MN frequencies in the control group were very low. The exposure situations were for both groups given as 7 h/d and (most probably) 5 d/week. The mean exposures were

factory: 0.21 ppm leading to a 2-week cumulative exposure of 14.7 ppm x h

laboratory: 0.28 ppm leading to a 2-week cumulative exposure of 19.6 ppm x h.

Ladeira et al. (2011): investigation on buccal cells in workers from histopathology laboratories. Although the authors state that MN determination followed the CBMN method (cytokinesis-block micronucleus) this procedure cannot be applied to measure MN in buccal cells. Most probably there was a substantial overlap of subjects studied by Ladeira with those of the study of Viegas et al. (2010). In both of the studies the same number of control subjects was used and the exposure levels were quite similar. Ladeira reported in 85 control subjects a frequency of 0.81‰ MN, but Viegas a very low value of 0.13‰. Interestingly in the study of Viegas the MN frequencies of the exposed pathology/anatomy workers (0.64‰) are slightly below and those of the factory workers (1.27‰) only slightly above the control value of the Ladeira study (0.81‰). This discrepancy between two studies of essentially the same group of investigators questions the reproducibility of the measurements. The foregoing study of Viegas et al. (2010) is not mentioned in the Lareida publication although many of the authors are involved in both studies. For calculation of the 2-week cumulative exposure the same exposure situations are taken as for the Viegas study: 7 h/d, 5 d/week. With a mean TWA of 0.16 ppm a cumulative exposure of 11.2 ppm x h is obtained.

Zeller et al. (2011): investigation on nasal cells in volunteers exposed over 5 days (4 h/d) at defined exposures of 0.3-0.7 ppm with peaks up to 0.8 ppm. Cumulative

exposure over the 5 days was 8.3 ppm x h. Cell sampling before and directly after last exposure and 1, 2 and 3 weeks thereafter. This sampling strategy should enable to capture the optimal time point according to the cell cycle of the oral mucosa. The MN frequencies pre-exposure served as control value.

In addition, the positive studies on local mutagenicity in the upper respiratory tract of exposed humans must also be interpreted in the light of a recent animal study. Exposure of rats up to 15 ppm for 4 weeks led as expected to a marked cell proliferation in the nasal tissues, but the rate of MN induction was not increased (Speit et al., 2011).

In conclusion, there are positive and negative studies for micronuclei in buccal or nasal cells of humans exposed to FA. These data are taken as supportive evidence in the CLH report for the classification of FA as carcinogenic cat 1A as stated on p. 11: “Indication of local genotoxicity in exposed humans as evidenced by increases in micronuclei frequency in buccal and nasal mucosa cells in several studies.”

Two independent reviews (BfR, 2006; Appel et al., 2006; Speit, Schmid, 2006) have questioned the reliability of the database on local MN induction in workers. In addition, the positive findings in humans are contradicted by an animal study with high, well defined exposures (Speit et al., 2011).

The most relevant negative studies are those of Speit et al. (2007a) (buccal cells) and of Zeller et al. (2011) (nasal cells). The number of exposed subjects is the highest in the Zeller study compared to other investigations on nasal cells. As regards buccal cells, the number of subjects in the Speit study compares well to the other studies apart from those of Viegas et al. (2010) and Lareida et al. (2011). Similarly, the peak exposures and the 2 week cumulative exposures of the Zeller and Speit studies are in the same range as the other studies, apart from Burgaz et al.

(2001, 2002) and Ye et al. (2005). But these latter studies gave only very crude exposure estimates relying on stationary measurements.

Overall the negative Speit and Zeller studies are well comparable to the other investigations reporting an increase of MN in nasal and buccal cells with regard to the number of subjects and the exposure situations.

An important advantage of the Speit and Zeller studies is the clearly defined exposure situation. Here the highly variable exposure concentrations and possible confounding by other substances at the workplace is excluded. There is good evidence that coexposure to other substances may have played a role from Ballarin et al. (1992) and Norppa et al. (1992) (wood dust) or from Burgaz et al. (2001) (different solvents).

It should be noted that some studies showed extremely high (Norppa et al., 1992) or low (Suruda et al., 1993; Viegas et al., 2010; Ladeira et al., 2011) MN frequencies in control samples and this may may indicate to problems with scoring in these studies.

Finally no dose response relationship was found by Ballarin et al. (1992) and Norppa et al. (1992) although their subgroups differed considerably with regard to the exposure concentrations. This may indicate to co-exposure to an unknown confounding substance.

Therefore, a weight of evidence assessment should put the emphasis on the studies of Speit et al. (2007a) and Zeller et al. (2011) carried out under strictly defined conditions. The positive studies on workers can therefore by no means be taken as sufficient evidence for the local mutagenicity of FA on the upper respiratory tract. These studies cannot be used as supportive evidence for a Cat.1 carcinogenicity classification.

The findings of mutagenicity in the upper respiratory tract in humans are not supported by an animal study with inhalation exposure up to 15 ppm.

SUMMARY

According to CLP regulation, section 3.6.2.2.5. there are several factors that "can be viewed as either increasing or decreasing the level of concern for human carcinogenicity."

In section 3.6.2.2.6. these factors are listed. The criteria a-f and h,I do not provide arguments for a cat.2 classification and the most important criteria to differentiate between cat. 1A and 2 for FA are:

g. structural similarity to a substance(s) for which there is good evidence for carcinogenicity

l. the possibility of a confounding effect of excessive toxicity at test doses

m. mode of action and its relevance to humans, such as *cytotoxicity with growth stimulation*, mitogenesis, immunosuppression, mutagenicity.

In vitro FA is clearly genotoxic and mutagenic leading predominantly to DPX formation and chromosome mutations in mammalian cell systems in the range of cytotoxic concentrations. There is evidence for a practical threshold for induction of micronuclei and SCE. FA after having entered a cell of primary contact is not passed on to neighbour cells.

After in vivo inhalation exposure exogenous FA leads to DPX formation, DNA adducts and DNA-DNA cross links only in nasal tissue of rats but not in organs remote from the site of first contact. DPX and DNA adduct formation have a highly non-linear dose response relationship. On the other hand, DNA adducts and DNA-DNA cross links have been identified stemming from endogenous FA in all tissues

investigated. Mutagenic effects have not been reliably demonstrated locally or systemically after inhalation exposure in experimental animals or humans.

FA leads to cytotoxic irritation with increased regenerative cell replication in the nose of exposed rats. A threshold has been demonstrated for the increase of cell replication at =/>2ppm in rats. This has to be seen in the context of efficient metabolic detoxification of FA in all tissues (biological half life of about 1 min); half saturation of the detoxification pathway was estimated to occur at exposure concentrations of 2.6 ppm.

Histopathological lesions are already induced in the nose of rats after a single day of exposure to the carcinogenic concentrations of 10 and 15 ppm. Extensive ulceration is already found after 4 days and squamous metaplasia after 9 days of exposure. Mild effects occur after a few days at 6 ppm. By prolonged exposure such lesions progress to hyperplasia and squamous metaplasia and finally to carcinomas.

There is a clear difference in the sensitivity of different species with regard to the carcinogenic activity of FA: mice are much less sensitive than rats and hamsters did not develop respiratory tumors after inhalation exposure. Furthermore, for the induction of cell proliferation and histopathological lesions much higher exposure concentrations are necessary for mice than for rats.

There is no reliable experimental or epidemiological evidence or a mechanistic basis for induction of systemic tumors.

These data lead to the conclusion that tumor development depends on excessive cytotoxicity leading to increased cell proliferation. Genotoxicity is of minor importance. Without such pronounced cytotoxicity and regenerative cell proliferation no tumors will develop. In addition, a carcinogenic effect of FA is only to be expected by inhalation exposure. The toxicological profiles of FA and acetaldehyde are

basically identical, apart from their potency, justifying the same classification for FA as that for acetaldehyde, namely cat. 2 under the CLP regulation.

In conclusion, apart from differences in species sensitivity, especially three of the criteria mentioned above need to be taken into consideration for a classification decision of FA, namely

g. structural similarity to a substance(s) for which there is good evidence for carcinogenicity

l. the possibility of a confounding effect of excessive toxicity at test doses

m. mode of action and its relevance to humans, such as *cytotoxicity with growth stimulation*, mitogenesis, immunosuppression, mutagenicity.

In humans tumors of the upper respiratory tract may only develop under conditions of high cytotoxicity with prolonged growth stimulation. Under these considerations FA was formerly classified as a category 3 carcinogen. Since that time no additional data have been reported that might call the former classification into question. Therefore these arguments also pertain to the new CLP regulation and would lead to category 2.

There are positive and negative studies for micronuclei induction in buccal or nasal cells of humans exposed to FA. These data are taken as supportive evidence in the CLH report for the classification of FA as carcinogenic cat 1A. A weight of evidence assessment showed that the negative studies of Speit et al. (2007a) and Zeller et al. (2011) carried out under strictly defined conditions are the most reliable ones to assess local mutagenicity in the upper respiratory tract of humans. The positive studies on MN induction in workers reported by other authors can by no means be taken as sufficient evidence for such a local mutagenicity. Therefore the overall database on MN induction in nasal or buccal cells in humans cannot be used as

supportive evidence for a Cat.1 carcinogenicity classification. In addition the negative studies in humans are supported by an inhalation study in rats not leading to MN formation in the nose at exposures up to 15 ppm.

Comments on the proposal to classify for mutagenicity cat. 2

Regulatory situation and general comments

Chapter 3.5. of the CLP regulation deals with deals with the classification for mutagenicity. Already in the heading it is made clear that this relates to “Germ cell mutagenicity”.

In 3.5.1. only the general definitions for mutation and genotoxicity are given and 3.5.2. relates to the classification criteria.

According to 3.5.2.1. “This hazard class is primarily concerned with substances that may cause mutations **in the germ cells** (emphasis added) of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class.” The second sentence means that in vitro test results or somatic cell data have also to be considered with respect to the potential that a substance may cause **germ cell mutations**.
The primary concern for germ cell mutagenicity if further underlined by 5.5.2.2. “For the purpose of classification for **germ cell mutagenicity**…“ and specifically also in the criteria for category 2: “Substances which cause concern for humans owing to the possibility that they may induce **heritable mutations in the germ cells** of humans (emphasis added).” It goes on that
“The classification in Category 2 is based on:

— positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
— somatic cell mutagenicity tests in vivo, in mammals; or
— other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays."

But this evidence clearly must be in line with the primary concern for germ cell mutagenicity.

Section 3.5.2.3. defines "Specific considerations for classification of substances as **germ cell mutagens**" and again this heading shows that this classification is related to **possible effects on germ cells**. In 3.5.2.3.1. test system to be considered are mentioned but the following parts make again clear that this relates to germ cells:
3.5.2.3.2. "The system is hazard based, classifying substances on the basis of their intrinsic ability to **induce mutations in germ cells** (emphasis added)."
3.5.2.3.3. "Classification for heritable effects in human **germ cells** is made on the basis of well conducted, sufficiently validated tests …(emphasis added)".
These criteria for germ cell mutagenicity have to be applied to FA taking into account that by its intrinsic high reactivity FA will only act on tissues of primary contact. The lack of systemic effects has been shown after long term inhalation and oral exposure and it has been substantiated by the most recent mechanistic studies: after inhalation exposure to labeled FA no increase of DNA adducts in tissues not at the site of first contact and no increase of the blood concentration stemming from the exogenous FA were found (the references are given in the sections above). Therefore, as FA does not reach distant targets apart from the site of first contact, germ cell effects (in the sense of mutagenicity or genotoxicity) after inhalation, oral or dermal exposure can be excluded and the basic criteria and the definition of germ cell mutagenicity are not met.

The restriction to germ cell mutagenicity is also valid when interpreting the "guidance document for CLP". In the section on "Classification as a Category 2 mutagen" it is

stated: „A Category 2 mutagen classification may also be based on positive results of a least one *in vivo* valid mammalian genotoxicity test, supported by positive *in vitro* mutagenicity results (p. 186)." This might be interpreted as pertaining to FA as its local genotoxicity has been demonstrated (e.g. DNA adduct formation at the site of first contact) as well as its in vitro mutagenicity, especially MN formation. But nevertheless it is made clear in the flow diagramm, that such effects must be interpreted in the light of germ cell effects (p. 189): "**According to the criteria, does the substance cause concern for humans owing to the possibility that it may induce** (emphasis added) heritable mutations in the germ cells of humans?"

Comments on specific studies

Although the toxicological profile does not warrant a classification for mutagenicity, in the following we want to comment on specific arguments given in the CLH report to support mutagenicity classification. These arguments are summarized on p.10, last paragraph:

"On mutagenicity, positive evidence are available i*n vivo* at the site of contact in somatic cells. They consist in induction of chromosomal aberrations in rats by inhalation at high dose (Dallas 1992) ….."

Regarding the Dallas study, further details are given on p. 82/83: "Besides, weak but positive genotoxic effects are observed such as the induction … of chromosomal aberrations in pulmonary cells at the highest dose of 15 ppm by inhalation (Dallas 1992). Compared to the OECD guideline, this latter study display no positive control and fewer cells were analysed than recommended (50 cells/animal instead of 100 in the guideline). However, these limitations were not considered to affect the validity of the study considering that a positive and statistically significant effect was observed at the highest dose in spite of the small number of cells analysed. … The recent study by Neuss *et al.* (2010a) also found no evidence of DPX in the modified Comet

assay and did not reproduce the induction of chromosomal aberrations in its micronucleus assay under experimental conditions comparable to Dallas *et al.* (1992). It should be noted that in Neuss 2010a the positive controls did not give an appropriate response for micronuclei induction. This study was performed according to a non-standard protocol that may explain why the standard positive control used in this assay is not appropriate in this case."

If the Dallas study is to be used as an argument for mutagenicity classification, it must be critically evaluated against the most recent negative study of Neuss (2010a). It is not sufficient to say that in the Neuss study "the positive controls did not give an appropriate response", as Dallas did not try a positive control substance at all, or that the Neuss study "was performed according to a non-standard protocol" as the same argument applies to Dallas and there is no standard protocol available for MN induction in lung lavage cells. So both studies have to be assessed on their own merits. Notwithstanding that both were "non-standard protocol" studies, in contrast to Dallas, the Neuss study was carried out under GLP conditions so that every finding can be verified.

One important point is the sensitivity and statistical reliability of both studies: the finding of Dallas rely on 50 cells each from 5 animals per group being scored (in total 250 cells) while Neuss investigated 2000 cell each from 6 animals per group (in total 12000 cells). Thus a chance finding in the Dallas study cannot be excluded when comparing the diverging findings of both investigations. In addition, in the Neuss study the lavage cells were investigated by the standard Comet assay (for DNA strand breaks and alkali-labile sites) as well as in a modified version with gamma irradiation (for DPX formation). For this part of the study MMS was used as positive control substance and led to the expected effect in the lavage cells. In contrast, no FA related effects were found in both of these comet assays, especially there was no increase in DPX, this lesion being the precursor of cytogenetic effects. This endpoint is not covered by Dallas.

One important point that sheds serious doubt whether the Dallas study was done according to nowadays state of the art can be derived from the discussion in the Neuss publication. Obviously the investigation published as Dallas et al. (1992) was already carried out in 1983 and reported as an abstract in 1985 (Scott et al., 1985). Only later in 1988 the rodent lung-macrophage chromosome assay was established in mice and Chinese hamsters. Thereby baseline aberration frequencies for mice of 1.2% and for hamsters of 0.75% were established. Another study is referenced by Neuss with a control aberration frequency in rats of 1%. The reason for the high control aberration frequencies of the Dallas study remains unknown, but is not in line with other more recent publications.

Dallas et al. themselves are very cautious in the interpretation of their results stating "however, the biological significance of this finding is uncertain. This is because the chromosome damage was marginal, it only occurred at a dose that is carcinogenic to the nasal cavities of rats, and there is no evidence that this dose of formaldehyde is carcinogenic to the lung of rats." At exposure concentrations of up to 15 ppm over 2 years no lung lesions were reported, but histopathological changes were confined to the nasal cavity and the proximal trachea (Kerns et al., 1983; Sellakumar et al., 1985). That FA does not reach the lung of rats was later indirectly confirmed by modeling the FA flux into the pulmonary regions of humans (Overton et al., 2001). Under the condition of rest that is comparable to the situation of rats in inhalation experiments, the FA flux virtually becomes zero in the region of the 10th bronchial generation (division of the conducting airways) and no FA will reach the pulmonary region beyond the terminal bronchioles. In addition it has to be taken into account that the rat nose more efficiently extracts FA from inhaled air as compared to that of primates: while FA at 6 ppm induced histopathological lesions in the trachea and bronchial bifurcation of monkeys (Monticello et al., 1989), such effects were not noted in rats at 5.6 ppm after chronic exposure (Kerns et al., 1983). And even in

monkeys no DPX (Heck et al., 1989) or histopathological lesions (Monticello et al., 1989) were found in the proximal lung.

In addition it is stated on p.10, last paragraph:
“On mutagenicity, positive evidence are available i*n vivo* at the site of contact in somatic cells. They consist in induction ….. of micronuclei in rats in the gastrointestinal tract by oral route (Migliore 1989).”
Regarding the Migliore study, it is stated on p. 82: “….weak but positive genotoxic effects are observed such as the induction of respectively micronuclei at irritating doses in the gastrointestinal tract via oral route (Migliore 1989)…..”.
In this study FA was given orally at a single high dose of 200 mg/kg and MN were analyzed 16, 24 and 39 h after treatment. MN were observed in the stomach and to a lesser extent in duodenum, ileum and colon. The frequency of MN increased with time after treatment. In addition other nuclear abnormalities indicative for cytotoxicity were observed in parallel to a high extent. It is noted that MN induction was clearly correlated with local irritation: hyperemia and hemorrhage. Maximal effects for MN induction and histopathological signs of severe irritation were both observed at the latest time of sacrifice, indicating that both effects were interrelated to each other. The effects obtained under this very high dose by bolus application can by no means be compared to inhalation exposure situations. Because of the exposure route and the high dose (only one dose level used not allowing to establish a dose response relationship) leading to severe irritation, these results should not be used for a regulatory mutagenicity classification of FA.

The classification cat.2 mutagenicity is said to be further supported by (p.10): “These positive data are further supported by *in vitro* positive results in numerous genotoxicity and mutagenicity tests, *in vivo* induction of DNA adducts and DNA-protein crosslinks (DPX) at the site of contact and indications of consistent increases

in micronuclei frequency in humans at the site of contact." To our interpretation of the CLP criteria and the corresponding guideline, these data do not support a classification for germ cell mutagenicity. We do not contest "*in vitro* positive results in numerous genotoxicity and mutagenicity tests", nor the ", *in vivo* induction of DNA adducts and DNA-protein crosslinks (DPX) at the site of contact", but the total database shows that these findings will not pertain to germ cells as FA does only act at the site of first contact, not reaching the germ cells by whatever route of exposure. The most recent data in this respect (no systemic DNA adduct or increase in blood concentration after exposure to labeled exogenous FA) have been given above. Finally, the "indications of consistent increases in micronuclei frequency in humans at the site of contact" have also been discussed above in detail. The positive studies from humans at the workplace and the negative studies in volunteers have been assessed in a weight of evidence approach. Thereby we showed that emphasis must be placed on the volunteer studies with exposures under strictly defined conditions.

Minor comments related to p.82/83:

"*In vivo*, at the site of contact, induction of DPX by inhalation was observed in rats in the nasal mucosa and in monkeys in the nasal turbinates and to a lower extent in the respiratory tract (Casanova 1991, Lu 2010, Lu 2011, Moeller 2011)." Lu et al. (2011) and Moeller et al. (2011) only measured DNA adducts and Lu et al. (2010) determined in addition DNA-DNA adducts but not DPX. The formation of DPX in the respiratory tract (apart from the nose) was found in monkeys but not in rats.

"Besides, recent studies able to discriminate between DNA-adducts of endogenous or exogenous origin shows that the level of exogenous DNA-adducts in rat nasal epithelium is of similar order of magnitude than endogenous DNA-adduct level up to 9 ppm but is dramatically increased at 15 ppm (Lu 2011)." If the dramatic increase at 15 ppm is mentioned, it should also be stated, that the increase of adducts by

exogenous FA at 0.7ppm amounted to about 1% of endogenous adducts and at 2 ppm to about 3%, clearly within the standard deviation of the endogenous adducts and is by no means “of similar order of magnitude than endogenous DNA-adduct level”. Only at 5.8 ppm the increase of 20% reached just the standard deviation of the endogenous adducts.

Finally it is mentioned that “DPX were found in the liver cells of mice from 0.8 ppm (Zhao 2009). Im *et al.* (2006) observed DNA damage in the Comet assay in the liver and lymphocytes from 5 ppm.” As these studies are obviously not taken as support for mutagenicity cat. 2 classification, they will only briefly assessed here. The results of both of these studies are in conflict with the most recent studies showing that exogenous FA will neither lead to an increase of blood levels nor to DNA adducts or DNA-DNA cross links apart from the site of direct contact. As regards specifically the findings of Im et al. (2006), Speit (2006) pointed in addition to the fact that even if FA had acted systemically the results observed in the comet assay are not biologically plausible. FA leads primarily to DPX formation and DPX reduce DNA migration in the comet assay and do not lead to an increase as reported by Im.

Summary

Classification for the different mutagenicity categories always refer to germ cell mutagenicity. Due to the high reactivity of FA, DPX, DNA adducts and DNA-DNA cross links have only been observed in the nasal tissue of rats after inhalation. Furthermore, inhalation of FA does not lead to an increase of its blood concentration. Therefore after exposure by the inhalation, oral or dermal route, FA will not reach the germ cells and a classification for (germ cell) mutagenicity is not warranted.

In the CLH report two studies are specifically mentioned as giving support to a mutagenicity cat. 2 classification:

Dallas et al. (1992) claimed that chromosomal aberrations in lung lavage cells sre induced after inhalation exposure. But this finding could not be reproduced in a recent inhalation study in which neither MN nor DNA strand breaks, alkali-labile sites or DPX were induced in lung lavage cells. This study carried out according to today's standards must be given precedence over the Dallas study. In addition, inhalation studies and mathematical modeling have shown the FA by inhalation will not reach the lung.

Migliore et al. (1989) reported MN formation in the gastric mucosa after oral application at a highly cytotoxic dose level leading to hyperemia and hemorrhage. This study can by no means be compared to the inhalation exposure situation.

In conclusion there is no reason to classify FA as a cat. 2 mutagen.

Route specific classification

Regulatory situation

According to Regulation (EC) No 1292/2008 (16 December 2008), "For the purpose of classification of health hazards (Part 3) **route of exposure** (emphasis added), mechanistic information and metabolism studies are pertinent to determining the relevance of an effect in humans." (section 1.1.1.5.).

And according to section 3.6.2.1: "route-specific classification may be warranted, if it can be conclusively proved that no other route of exposure exhibits the hazard".

And according to section 3.6.2.2.8: "The classification shall take into consideration whether or not the substance is absorbed by a given route(s); or whether there are only local tumours at the site of administration for the tested route(s), and adequate testing by other major route(s) show lack of carcinogenicity."

Not much additional guidance in this respect is given in the guidance document for CLP classification apart from (p.203/204):" The classification for carcinogenicity generally does not specify specific routes of exposure. …. However, under the previous EU system (Annex VI to DSD), classification specifically via inhalation was accepted by application of the risk phrase R49; May cause cancer by inhalation and a specific hazard statement has been established in CLP, H350i; May cause cancer by inhalation (CLP, Annex VII, Table 1.1). The hazard statement allows for identifying the route of exposure "if it is conclusively proven that no other routes of exposure cause the hazard" (Annex I, section 3.6.4.1). In this case the hazard statement may be modified accordingly. Genotoxic carcinogens are generally suspected to be carcinogenic by any route."

Regarding the last sentence it has to be taken into account that the genotoxic activity of FA is strictly confined to the site of its primary contact, i.e. the respiratory tract after inhalation. Studies showing systemic genotoxicity cannot be taken as reliable.

We propose that a classification of FA should be limited to the inhalation route. In this respect it will be discussed

- Are reliable carcinogenicity bioassays available for the oral and dermal route?
- Are genotoxic effects to be expected for these routes?
- Are mechanistc data available that support to confine classification to the inhalation route only?

No classification for the oral route

Til et al. (1989) exposed rats over 2 years to FA via drinking water (up to 1900 mg/l). The major effects observed were severe lesions in the forestomach and in the glandular stomach most likely due to the corrosive properties of FA, kidney lesions mainly ascribed to dehydration (reduced water uptake) and a yellow discoloration of the fur. Neither local nor systemic tumors were observed. The yellow diyscoloration of the fur was ascribed to direct contamination of the fur by FA spoiled from the drinking water bottles (Til et al., 1988).

Takahashi et al. (1986) described papillomas in the forestomach of rats exposed to 0.2% FA in the drinking water after 32 weeks. These lesions were accompanied by erosions or even ulcers, penetrating as far as into the base of the mucosa, along the limiting ridge of the fundic mucosa. If FA was given after initiation with MNNG and sodium chloride, preneoplastic and neoplastic effects were found in the pyloric region of the glandular stomach. The diverging findings after treatment with FA alone between the Til and Takahashi studies were discussed by Til et al. (1989). They proposed that the differences might either be ascribed to different sensitivities of the rat strains used to the irritating effects of FA, or to different criteria for the classification of a lesion as papilloma or hyperplasia. A clear description of the histopathological criteria to differentiate between papilloma and papillary epithelial hyperplasia as given by Til is not found in the Takahashi publication. But regardless of these interpretations, the papillomas in the Takahashi study only occurred in parallel to massive tissue destruction accompanied by regenerative cell replication. Similarly, the findings after application of FA as promotor were ascribed by the authors to gastric irritation and damage to the mucous membrane.

Soffritti et al. (1989, 2002) reported the induction of systemic tumors and indications for local tumor induction after oral exposure of rats to FA. We agree to the assessment of the CLH report that these studies are not reliable and therefore they will not be discussed any further in this context.

Supporting evidence that the carcinogenic action of FA is specific to the inhalation route may be derived from data for acrolein. This substance with a double bond in conjugation to the carbonyl group is highly reactive to nucleophilic substances. This explains its severe local irritation and its genotoxicity (among others leading to DNA adducts in vivo) similar to FA. Two carcinogenicity studies with acrolein are described by MAK (1997): rats were exposed orally to 0.05, 0.5, and 2.5 mg/kg bw by gavage over 102 weeks and mice to 0.5, 2, and 4.5 mg/kg bw over 18 months. At the highest dose levels early mortality was noted, thus the MTD was reached. In both species neither local nor systemic tumors were found.

Similarly, there are two drinking water studies available for glutaraldehyde, one with Wistar and one with F344 rats, at dose levels of 50, 250 and 1000 mg/l. The combined data of both studies show that glutaraldehyde does not exhibit a carcinogenic effect, neither locally nor systemically (MAK 2002, 2006).

In conclusion, a carcinogenicity classification of FA after oral exposure is not warranted for the following reasons:

- No tumors were observed in a guideline 2-year carcinogenicity bioassay
- Indications for the development of forestomach papillomas in another study are uncertain due to the unclear histopathological criteria applied
- No indications for tumor development were obtained after oral application of two other highly reactive aldehydes.

No classification for the dermal route

Three initiation/promotion studies on the skin of mice are available and referenced in the CLH report (Spangler, Ward, 1983; Krivanek et al., 1983; Iversen, 1986). FA did

not act as an initiator or promotor. All these studies also included a group of FA-only treated mice and neither systemic nor local tumors were found. But these results with FA-only cannot be taken to reliably exclude a carcinogenic activity of FA via skin application regarding exposure duration, number of experimental animals or details for dose selection:

- Sprangler, Ward: 30 female Sencar mice, 1 or 2 times/week over 48 weeks, 3.7% FA solution
- Krivanek: 30 female CD-1 mice, 3 times/week over 36 weeks, once a 10% and then 1% solution of FA
- Iversen: 16 Oslo hairless mice/sex; 2 times/week over 60 weeks; 1% and 10% FA solutions

Only Krivanek carried out a pretest and defined the concentrations used in his study as slightly irritating.

No definitive answer regarding a carcinogenic effect of FA following skin application can be derived from these studies. On the other hand, mechanistic considerations clearly indicate that neither a local nor a systemic activity of FA is to be expected by this route of exposure. It has been shown above (Neuss and Speit, 2008; Neuss et al., 2010) that FA after having entered the first cell of contact is not passed on to the neighbour cells. Taking into account the multi-layered epithelium of the skin, FA will not reach the dividing basal cells of the skin to exert here its cytotoxic or genotoxic activity. The basal cells could at most only be affected by repeated application of concentrations leading to deep necrosis. Similarly, a systemic carcinogenic activity can be excluded. Although Jeffcoat et al. (1983) and Bartnik et al. (1985) found systemic distribution of radioactivity in the body of experimental animals, this cannot be taken as evidence that unmetabolised FA may enter the body. These authors

applied radioactive FA to the skin and only measured radioactivity in the body without specifically analysing for FA. As not even after inhalation exposure unmetabolised FA or its DNA adducts were found in tissue far off the port of entry this can also be excluded for dermal exposure.

In conclusion, in experiments with skin application FA did not act as initiator or promotor. Although treatments with FA alone were included in these experiment, a definite answer as to whether FA may be a complete skin carcinogen is not possible by these data. But as FA will only act on cells of its primary contact and taking into account the multilayered structure of the skin, a local carcinogenic activity can be excluded.

Mannheim, Essen, Dec. 10, 2011

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