

FORMALDEHYDE TOXICOLOGY - SCIENTIFIC UPDATE INFORMATION **(September 2007)**

Several new studies specifically designed to examine formaldehyde's toxicological properties have been initiated in the last three years, a number of them have been finalised in the meantime. These studies aim to support regulators at national and European level to arrive at a balanced evaluation of formaldehyde's impact on human health. This update gives an overview of the scientific evaluation and lists and explains the latest results.

Background

Available data shows that a connection between formaldehyde exposure at current levels of exposure and cancer in humans is unlikely. Yet doubts exist about formaldehyde's role in a rare form of cancer affecting the upper respiratory tract.

Formaldehyde, an essential metabolic intermediate in all living cells, is used in a wide range of applications, including building materials, furniture, paints and coatings, textiles and pharmaceuticals. In June 2004, IARC¹ recommended that formaldehyde should be regarded as an IARC Group 1 carcinogen. This recommendation is not legally binding. In the preamble to monographs it is explicitly emphasized by the IARC that, "no recommendation is given with regard to regulation or legislation". At European level, the IARC recommendation triggered a review of formaldehyde's existing category 3 classification, the lowest available EU category for suspected carcinogens.²

IARC primarily bases its 2004-recommendation (that was only published with a delay of more than 2,5 years in January 2007) on one epidemiological study, the so-called NCI³/Hauptmann study, which showed a slight excess in the total number of nasopharyngeal cancers (NPC) among workers exposed to formaldehyde. The NCI study did not take into account the fact that over half of the NPC cases observed in the NCI study originated from one single plant in the US. This suggests that factors other than formaldehyde exposure may have been at play.

Scientific Data

- It has been known for already 25 years that formaldehyde at highly irritating concentrations may lead to **nasal tumor development in rats**. Tumor development was confined to concentrations leading to strong cytotoxic irritation and increased cell proliferation in the nose of rats. Such effects are today considered to be the prerequisite for later tumor formation.
- **Over 50 epidemiological investigations** on possible cancer development in formaldehyde exposed workers have been carried out. These studies have been reviewed periodically by different authorities and scientific organisations. **Up to 2003 the general conclusion** was that the overall strength of **evidence did not show a clear and**

¹ International Agency for Research on Cancer, Lyon, France

² Criteria given in the Dangerous Substances Directive - category 3 (C3) carcinogen are assigned the risk phrase R 40 (Limited evidence of a carcinogenic effect). This is the weakest class of carcinogenic hazard.

³ NCI: National Cancer Institute, Rockville, Maryland, USA

causal association between formaldehyde exposure and tumor development in humans. This was also the conclusion of repeatedly convening expert working groups at IARC in 1982, 1987 and 1995.

- **In 2004 a new expert group of IARC** concluded that there is sufficient evidence of carcinogenicity in humans. This was mainly based on the recent epidemiological updates of one of the **three largest cohort studies** in formaldehyde exposed workers.. This study evaluated 25,500 workers out of a total of nearly 50,000 workers with exposures dating back up to 1937 in all 3 studies.
- Two tumor types were of major concern for IARC:
 - **Nasopharyngeal Cancer (NPC):** This is a very rare tumor type. Only in one of the three cohort studies, the one (NCI) that evaluated 25,500 workers, an increase of nasopharyngeal cancer was found by ten cases of this tumor type. Two of them occurred in workers not exposed to formaldehyde, eight in the formaldehyde exposed workers which is about twice as much as expected. In conjunction with positive findings from some other studies that were by far not as comprehensive as the three large studies mentioned above, the IARC working group concluded that this "provided sufficient epidemiological evidence". It should be noted that most of the cancer cases of this first study came from **one specific plant** while no such increase was noted in any of the other nine plants of this investigation. A detailed analysis of this one plant led another investigator to the conclusion that other factors (especially silver smithing or metal work) other than formaldehyde exposure might have caused this increased tumor incidence. In addition, the other two epidemiological updates comprising approximately 25,000 workers did not show an increase in nasopharyngeal tumors similar to the total of 50,000 workers in all of the three large studies.
 - **Leukemia:** IARC concluded that "there is strong but not sufficient evidence for a causal association (with) occupational exposure to formaldehyde". In some studies an increase in leukemia has been observed in professional workers like embalmers, pathologists or anatomists. In addition, there was a statistically significant increase for workers exposed to high levels of formaldehyde in the update of the large study with 25,500 workers and in one of the other two recent updates (the NIOSH study). However, in the NCI study leukemia mortality was only increased in internal comparisons due to an extremely low leukemia mortality in unexposed workers (SMR=0.38), but not if compared to the general population (SMR=0.85). A significant association for all leukemias was only observed for peak, but not for average, cumulative or duration of exposure. Only for myeloid leukemia a significant association was also found with regard to average intensity of exposure. In contrast, in the NIOSH study significant associations were only found in the subgroup of workers with 10 or more years of exposure or / and 20 years or more since first exposure.

Therefore, FormaCare seriously doubts that the epidemiological data are sufficient for a carcinogen category 1 classification in the EU which requires "sufficient evidence to establish a causal association between human exposure to a substance and the development of cancer".

Unlike the epidemiological data on formaldehyde, at the time of the last IARC evaluation substantial new toxicological data had not been developed since the mid 90s. For example, much of the literature on human eye and nose irritation is old and not very robust, but valid data on these endpoints would be pivotal for setting exposure limits. The relationship between DNA-protein crosslinks, the predisposition of the affected genomes to mutate and cell toxicity is unclear. The methodology used to measure the in-vivo development of

micronuclei (a marker of mutagenesis) in cells of the upper respiratory tracts is not very reliable. In order to clarify these crucial questions, FormaCare initiated several new studies to generate new epidemiological and toxicological data on formaldehyde.

Below, the current and newly published scientific study results on formaldehyde's impact on human health are described in summary. They cover four areas:

1) Epidemiology.

- A literature review on possible risk factors for NPC carried out by **Professor Adami** (Karolinska Institute, Stockholm, Sweden) and **Dr Chang** (Northern California Cancer Center (NCCC), Fremont, California, USA) showed that other factors (e.g. a specific genetic configuration, or even a particular diet) may lead to an increased risk by a factor of ten. They concluded that the epidemiological evidence for a causal association between formaldehyde and NPC is limited.
- After IARC evaluated formaldehyde, new analyses of the same data used by NCI were published in 2005 (**Professors Marsh/Youk**, University of Pittsburgh, Pennsylvania, USA) which show that there is considerable uncertainty whether the human evidence for nasopharyngeal cancer identified by IARC is related to formaldehyde exposure.⁴
- It could recently be demonstrated that the statistics used in the NCI/Hauptmann study have significant shortcomings. Sensitivity and influence analyses carried out by **Professor Marsh** (University of Pittsburgh, Pennsylvania, USA) and **Dr Morfeld** (University of Cologne, Germany) clearly indicated a lack of statistical robustness of the original NCI analysis.
- In a most recent publication on the specific plant with the highest incidence of NPC Gary Marsh came to the conclusion that previous occupational exposure to silver smithing or other metal work might have been an important factor contributing to the development of this specific tumor.
- Ten more years of epidemiological follow-up of the NCI study will become available in 2008. This so-called **NCI update** integrates the latest available data to cover the period from 1994 (date of the last investigation) until 2004.⁵

2) The genotoxicity and mutagenicity of formaldehyde.

- A literature review by **Professor Speit** (University of Ulm, Germany) shows that:
 - the local genotoxic effects reported for cells in the mouth and nose of humans are not consistent
 - there is a lack of standardization of the method
 - there is often incomplete reporting as regards study design, exposure, or confounders.
- In conjunction with **Professor Triebig's** (University of Heidelberg, Germany) investigations with human volunteers, buccal cells were taken and tested for

⁴ This is not a FormaCare study.

⁵ This is not a FormaCare study.

mutations (micronuclei) by **Professor Speit**. In conclusion, formaldehyde did not lead to an increase in micronuclei formation under conditions simulating workplace exposure situations.

- Experiments within cell lines comparing the dose response curves and thresholds for formaldehyde induced DPX, mutational events, and cytotoxicity show that DPX formation occurs at lower concentrations than true mutations or cell toxicity (**Professor Speit**). Furthermore, DPX are rapidly repaired and there was indication for a threshold for the induction of mutations. Similar comparisons for nasal tissues in rats after inhalation are in progress.
- Experimental determination of genotoxic events in human lymphocytes *in vitro* from **Professor Speit** show that a good repair mechanism for DPX in lymphocytes from human blood exists. It was also determined that the sensitivity of DPX formation was highest, followed by cytotoxicity, SCE and finally micronuclei formation. The conclusion is that a mutagenic effect of formaldehyde on lymphocytes after inhalation (as a first step to leukemia) is unlikely.
- Similar research with cell lines derived from human cells of the mouth is currently carried out and the final results are expected later in 2007. (**Professor Grafström**, Institute of Environmental Medicine Division of Biochemical Toxicology and Experimental Cancer Research, Karolinska Institute, Stockholm, Sweden).
- A three week inhalation study in rats was conducted by the CIIT to provide initial information on changes in gene expression in target areas and to associate these changes with overt tissue damage. A highly concentration and time-dependent response was noted. The results provide strong biological support for a biological threshold for formaldehyde-related effects. A more robust investigation (i.e., 90-day subchronic study) is under way.

3) The threshold for irritation in humans.

- A 2006 publication by **Dr Arts** (CIVO Centraal Instituut voor Voedingsonderzoek - and TNO - Nederlandse organisatie voor Toegepast-Natuurwetenschappelijk Onderzoek, Zeist, Netherlands) in the Journal of Regulatory Toxicology and Pharmacology suggests only minor/negligible irritation on the eyes and nose of humans at concentrations of less than 1 ppm.
- In order to define human sensory irritation and odour perception in relation to formaldehyde exposure more clearly, an experimental study was carried out by **Professor Triebig** at the Institute and Out-patient Clinic of Occupational and Social Medicine of the University of Heidelberg. The results demonstrate that objective signs of irritation do not occur at exposure levels up to 0.3 ppm plus peak exposures of 0,6 ppm or 0.5 ppm continuously.

4) Leukemia

A histopathology re-evaluation was carried out by an independent international group of pathologists, led by **Dr Ruud Woutersen** (TNO Quality of Life/Toxicology & Applied Pharmacology, Zeist, Netherlands). The largest chronic inhalation animal carcinogenicity study for which original data and slides are still available was re-evaluated specifically for leukemia formation. In rats no association between formaldehyde inhalation and leukemia was found. In mice there was an indication for treatment related occurrence of lymphomas and the relevance of this finding currently is under further investigation.

More details about the studies

1) Epidemiology:

A literature review published in 2006 on possible risk factors for NPC carried out by **Professor Adami** (Karolinska Institute, Stockholm, Sweden) and **Dr Chang** (Northern California Cancer Center, Fremont, USA) showed that there are several factors that may substantially increase the risk of formation of NPC: while workplace exposures may not be a predominant factor, other reasons, such as the Epstein-Barr-Virus, a specific genetic or familial configuration, or even a particular diet (e.g. salted fish) can lead to an increase of NPC by a factor of up to ten. The authors further concluded that the epidemiological evidence for a causal association between formaldehyde and NPC is limited.

After IARC evaluated formaldehyde, new analyses of the same data used by NCI were published in 2005 (**Professors Marsh/Youk**, University of Pittsburgh, Pennsylvania, USA) which showed that there is considerable uncertainty whether the human evidence for nasopharyngeal cancer identified by IARC is really related to formaldehyde exposure.

Furthermore, a study carried out for FormaCare demonstrated that the NCI/Hauptmann study has significant shortcomings:

Sensitivity and influence analyses carried out by **Professor Marsh** (University of Pittsburgh, Pennsylvania, USA) and **Dr Morfeld** (University of Cologne, Germany) shed serious doubt on the NCI data's statistical robustness. They come to the conclusion that the results of the analysis of nasopharyngeal cancer (NPC) in the NCI-cohort published by Dr Hauptmann et. al. (2004) are misleading because they are based on inappropriate regression analyses.

In the three large cohort studies mentioned above overall 50 000 chemical workers have been investigated who were in contact with formaldehyde at the workplace. Nine nasopharyngeal cancer cases have been registered – seven would have been expected for the general population; a significant exposure-related effect cannot be deduced from these numbers. Furthermore, most of the NPC came from one single plant of the ten investigated in the NCI/Hauptmann study.

In a recent epidemiological investigation of this specific plant with a detailed exposure analysis including pre-employment times the group of Professor Marsh concluded that co-exposure by silver smithing and other metal works should be considered as underlying causes for NPC.

The **NCI update** will add ten more years of epidemiological data to the latest NCI study. According to the latest communication by NCI, final manuscripts will be available by 2007/2008. Of course this is not a FormaCare study.

2) The genotoxicity and mutagenicity of formaldehyde

For the assessment of formaldehyde-carcinogenicity the question of genotoxicity and mutagenicity in target tissues is pivotal. Several studies have been commissioned by FormaCare in order to find out if a clear threshold for cancer development can be applied. Studies carried out in this respect come up with the following results:

- a) Review of published literature on local micronuclei formation
An increased incidence of micronuclei in nasal and buccal epithelial cells has been reported by several authors in literature for formaldehyde exposed humans. The published studies have been critically reviewed by **Professors Speit** and **Schmid** (“Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells”; is published in Mutation Research). The evaluation of in total 8 studies revealed that
- the local genotoxic effects reported are not consistent
 - there is a lack of standardisation of the method
 - there is often incomplete reporting as regards study design, exposure, or confounders.
- b) Experimental determination of micronuclei formation in buccal cells
Because of these inconsistent literature data, micronuclei were determined by **Professor Speit** of the University of Ulm in buccal cells harvested from the volunteers participating in the irritation study by **Professor Triebig**. In conclusion, formaldehyde did not lead to an increase in micronuclei formation when comparing pre- and post-exposure micronuclei frequencies.
- c) Experimental determination of dose response curves and thresholds for formaldehyde induced DPX, mutational events, and cytotoxicity
With regard to the question whether formaldehyde-induced carcinogenicity is driven by genotoxicity (or rather by cytotoxicity) the dose-response curves and relationships of formaldehyde-induced DNA-protein crosslinks (DPX) and mutational events are pivotal. *In vitro* investigations by **Professor Speit** led to the conclusion that
- DPX-formation is the most sensitive endpoint while “true” mutations (micronuclei formation) require higher formaldehyde concentrations
 - at low concentrations the dose-response curves are clearly upward-bent and not linear indicating to a threshold for mutations
 - DPX lesions are rapidly repaired and an accumulation is highly unlikely
 - There is good indication that DPX repair is even accelerated by repeated formaldehyde exposures.
- d) Experimental determination of genotoxic events in human lymphocytes *in vitro*
Similar *in vitro* experiments have been carried out with human blood lymphocytes. In conclusion, these studies show that a good repair mechanism for DPX in human lymphocytes exists. It was also determined that the sensitivity of DPX formation was highest followed by cytotoxicity, SCE and finally micronuclei formation. **Professor Speit** concludes that mutagenic effects of formaldehyde are very unlikely to occur in peripheral blood of exposed subjects. These findings also relate to the question whether there may be a plausible causal relationship between leukemia formation and formaldehyde exposure and refute such an assumption.
- e) Studies with rats to support biological-based cancer modelling
A number of studies have been commissioned by the US’s Formaldehyde Council to the CIIT in North Carolina.

In the first experiment, animals were exposed to formaldehyde at 0/ 0,7/ 2/ 6 or 15 ppm, respectively, for up to 15 days. Nasal tissues were taken at various times and examined for histopathological lesions, cell replication, and changes in gene expressions (genomics). This study was designed to apply genomic data along with cancer model to estimate risks below overt toxic concentrations.

The second experiment consists of a 90-day inhalation study that will be carried out to assess gene changes, cellular changes and more robust nasal epithelial cell proliferation kinetics following exposure to similar doses (0.7 – 15 ppm) used in the original rat bioassay study.. The results are expected to support the CIIT clonal growth risk assessment model for formaldehyde, understand the low-dose cell proliferation response that may be “J” shaped, and may also provide further support for a biological threshold for tumor formation. This study was initiated in the 1st quarter 2007 and results will be available 2007/2008.

f) Ongoing studies

Further studies on the relationship of genotoxicity / mutagenicity / cytotoxicity are still ongoing and results are not yet available:

- *in vivo* investigation of local mutagenicity and cytotoxicity in nasal mucosa of rats exposed to formaldehyde by inhalation (Speit, results expected beginning 2008): Rats will be exposed to formaldehyde. Cell replication and the formation of micronuclei in the rats' respiratory epithelium will be measured, a procedure that involves the development of a new method of evaluating the micronuclei in nasal tissue. This study of **Professor Speit** will define dose-response curves and thresholds for DPX (taken from literature), micronuclei formation, and cytotoxicity and put these effects quantitatively into context with each other. Results are expected in the first quarter of 2008.
- *in vitro* investigations with keratinocytes (Grafström, results expected by end of 2007): Keratinocytes are a potential target cell population in the upper respiratory tract, e.g. the nasopharyngeal region. The relationship between the formation of DNA-protein crosslinks, potential mutagenicity, and cytotoxicity for such cell types is unclear. FormaCare therefore supports a study from **Professor Grafström** (Stockholm's Karolinska Institute) to evaluate the *in vitro* dose response curves and thresholds for genotoxicity, mutagenicity and cytotoxicity in human keratinocytes and cell lines derived thereof.

3) The threshold for irritation in humans

In consideration of the mechanistic data the German MAK⁶ Commission assigned formaldehyde to Category 4 of carcinogenic substances which means that under the conditions of the MAK value “no significant contribution to human cancer risk is expected” at

⁶ MAK Value: "Maximale Arbeitsplatzkonzentration", German occupational exposure level developed by the respective German expert commission.

the workplace. The workplace exposure limit has been set by the MAK commission at 0,3 ppm.

Furthermore, a biological/mathematical model was developed in the US using the mechanistic data and the airflow in the upper respiratory tract of different species. By this model the cancer risk for a continuous life-time exposure (80 years) to formaldehyde at 0.1 ppm was calculated to be approximately five additional tumor cases for one million smokers or three additional tumor cases for 10 million non-smokers.

Data shows that cytotoxic irritation (irritation of the cells leading to cell destruction) can be a step towards cancer development. As for ethical reasons cytotoxic irritation may not be investigated in humans, a study was initiated to explore at which formaldehyde concentrations sensory irritation in humans sets on. It has to be stressed that cytotoxic irritation will occur at higher concentrations than sensory irritation.

A literature review on eye and upper respiratory tract irritation in humans by **Dr Arts** (TNO-CIVO) has been published by the Journal of Regulatory Toxicology and Pharmacology. Dr Arts' concludes that "sensory irritation is first observed at levels of 1 ppm and higher" and that "at airborne levels for which the prevalence of sensory irritation is minimal both in incidence and degree (i.e. < 1ppm), risks of respiratory tract cancer are considered to be negligibly low." But the data available for that review stem from old studies that cannot be considered scientifically robust according to nowadays' standards.

FormaCare thus supported a study by **Professor Triebig** from the University of Heidelberg to reliably define thresholds for eye and nose irritation applying modern methods. This very recent state-of-the-art sensory irritation study found objective signs of irritation only with an exposure of 0.5 ppm plus peak exposures of 1 ppm, but not for exposures of 0.5 ppm continuously or 0.3 ppm plus peaks of 0.6 ppm. The study will be published in a Peer Review Journal end of 2007.

4) Leukemia

While according to IARC the epidemiological studies have given an indication for leukemia formation in humans (but short of a category 1 classification), inhalation studies in rats, mice and hamsters have not reported such an effect. In a well designed drinking water study in rats there was no indication for induction of leukemia, but another study did report such effects. However, in this latter investigation major methodological problems and inconsistencies were noted by the IARC working group.

In order to check for leukemia formation in animals at very high experimental inhalation concentrations, the materials available from the largest animal study at CIIT were re-investigated led by **Dr Ruud Woutersen** (TNO Quality of Life/Toxicology & Applied Pharmacology). Thereby no treatment related leukemia was found in rats, but in mice there was an equivocal result for lymphoma after formaldehyde exposure. The meaning of this latter finding is under further investigation

In the U.S. **Dr Swenberg** (University of North Carolina) is conducting a study to determine the degree of formaldehyde interaction with DNA in the nose and internal organs from external formaldehyde exposure and internally (metabolically) generated formaldehyde. The research aims at establishing whether or not formaldehyde induces DNA-DNA cross-links at the site of contact and/or at distant tissues. Data from the research will provide critical

evidence related to the biological plausibility that formaldehyde does or does not have the potential to cause leukemia. Results are expected by 2007/2008.

About FormaCare

As a sector group of Cefic (the European Chemical Industry Council), FormaCare represents key European producers of formaldehyde, aminoplast glues and polyols. FormaCare aims to promote the sustainable use of formaldehyde and formaldehyde based products among its members and their customers, with due regard to health and environmental care.

Contact

FormaCare secretariat:

tel.: +32 2 676 72 67

fax : +32 2 676 73 59

formaldehyde@cefic.be

www.formacare.org