Subchronic (13-week) inhalation toxicity study of formaldehyde in male rats: 8-hour continuous versus 8-hour intermittent exposures

een onderzoek
Uitgevoerd in opdracht van het Directoraat-Generaal van de Arbeid
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Auteurs:
Dr. J.W.G.M. Wilmer
Dr. R.A. Woutersen
Drs. L.M. Appelman

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AUTHENTICATION

We, the undersigned, hereby declare that this work was performed under our supervision, according to the procedures herein described, and that this report represents a true and accurate record of the results obtained.

Dr J.W.C.M. Wilmer
Genetic toxicologist/deputy study director

date : October 31, 1986

Dr R.A. Woutersen
Pathologist

date : october 31, 1986

Drs L.M. Appelman
Study director

date : October 31, 1986

Dr V.J. Feron
Head, Department of Biological Toxicology

date : October 31, 1986

TNO QUALITY ASSURANCE UNIT (TOXCICOLOGY)
P.O. BOX 360, 3700 AJ ZEIST, the Netherlands

STATEMENT OF GLP COMPLIANCE

On : Subchronic inhalation toxicity study with formaldehyde in rats: 8-hour continuous versus 8-hour intermittent exposures
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The study was carried out under conditions of good laboratory practice. Within reason there have been no circumstances that might have affected the quality and integrity of the results obtained.

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Drs S. van Straten
Quality Assurance Manager

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SUMMARY

1. A subchronic (13-week) inhalation toxicity study with formaldehyde was carried out in male rats. The aim of the study was to find out whether excursions of the exposure concentration by a factor 2, under conditions of concentration x time (C x T) is constant, visibly affect the cytotoxic action of formaldehyde on the nasal epithelium.
   Four groups of rats (initial number: 25 per group) were continuously exposed to 1 or 2 ppm formaldehyde (8 hours a day), or interruptedly to 2 or 4 ppm (8 successive 1 h periods a day, each consisting of 30 min of exposure and 30 min of non-exposure), 5 days a week for 13 weeks. A control group was exposed to fresh air only. At the end of the 13-week experimental period, 10 animals of each group were injected with [^{3}H]thymidine to study the cell turnover in the nasal epithelium. In addition, 5 satellite groups of 5 rats each (5 per control group and 5 per each test group) were killed after a treatment period of 3 days and were used to study the cell turnover in the nasal epithelium.

2. Exposure to formaldehyde did not influence the behaviour and appearance of the animals.

3. As compared to the controls no differences in body weights were observed in the test groups.

4. No increase in the number of [^{3}H]thymidine labeled cells of the nasal respiratory epithelium was found in rats exposed to formaldehyde on 3 consecutive days. At the end of the 13-week exposure period, the cell turnover rate was higher in the group interruptedly exposed to 4 ppm formaldehyde than in controls.

5. Gross examination at autopsy did not reveal treatment-related pathological changes.

6. Treatment-related histopathological changes were only found in the nose of animals interruptedly exposed to 4 ppm formaldehyde. They comprised:

(a) increase in degree and incidence of disarranged respiratory epithelium lining the septum and the nasoturbinates

(b) increased incidence of squamous metaplasia of the respiratory epithelium frequently accompanied by basal cell hyperplasia and occasionally by keratinisation.

9. Two ppm formaldehyde was a non-toxic effect level in the present study.

10. It was concluded that under the conditions used in the present study (subcytotoxic and marginally cytotoxic exposure levels and a relatively long treatment-period) the exposure concentration rather than the total dose is primarily responsible for the adverse effects of formaldehyde on the nasal epithelium.

SUBCHRONIC (13-WEEK) INHALATION TOXICITY STUDY OF FORMALDEHYDE IN MALE RATS: 8-HOUR CONTINUOUS VERSUS 8-HOUR INTERMITTENT EXPOSURES

1. INTRODUCTION

Swenberg et al. (1983) have shown that the degree of cell proliferation in the nasal respiratory epithelium depends on the exposure concentration rather than on the total dose of formaldehyde. Their experiments were carried out with several groups of rats each receiving the same cumulative dose of 36 ppm.h formaldehyde by inhalation but after exposure to different concentrations and for different periods of time (3 ppm x 12 h, 6 ppm x 6 h and 12 ppm x 3 h). Wilmer et al. (1986) carried out a 4-week inhalation study in male rats which were exposed to 0, 5 or 10 ppm formaldehyde continuously (8 hours a day) or to 10 or 20 ppm formaldehyde interruptedly (8 successive 1 h periods a day, each consisting of 30 min of exposure and 30 min of non-exposure). They found that also under these experimental conditions (concentration x time is constant and repeated exposures during 4 weeks), the exposure concentration rather than the total dose is decisive of the severity of the cytotoxic effect of formaldehyde on the nasal epithelium. However, in this 4-week study relatively high, clearly cytotoxic formaldehyde concentrations were used. The question arose whether the aforementioned conclusion is also valid when lower exposure levels (marginally cytotoxic for the nasal mucosa of rats) and a longer exposure period are used. Therefore, a 13-week rat study similar in design and conduct to the previous 4-week study but using exposure concentrations of 1, 2 and 4 ppm formaldehyde was carried out. This 13-week study is described in the present report.

2. MATERIALS AND METHODS

2.1 Materials

Paraformaldehyde was purchased from Janssen Chimica, Beerse, Belgium.

[Methyl-^{3}H]thymidine (specific activity: 1.48 TBq/mmol) was obtained from Amersham International, Buckinghamshire, England.

2.2 Animals

One hundred and fifty male albino SPF Wistar rats (Cpb: Wu, Wistar random) were obtained from TNO Central Institute for the Breeding of Laboratory Animals, Zeist, the Netherlands. On arrival the rats were 4 weeks of age and weighed 40 to 60 g. The rats were checked for overt signs of ill health and anomalies. The animals were assigned by computer randomization to 5 groups of 25 males each and to 5 satellite groups of 5 males each to be used in a 3-day cell proliferation study.

2.3 Exposure chambers

The animals were exposed to the test substance in horizontally placed glass cylinders (0.90 x 0.15 m) with sampling ports at the inlet and the outlet part of the cylinder. Each cylinder was fitted with an interior of perforated stainless steel plate for individual housing of the animals. The total air flow through each cylinder was 20 l/min. The cylinders were kept at a temperature of about 20 °C.

2.4 Maintenance

During the exposure periods and the 30-minute intervals the test and control animals were housed in the glass cylinders. After each exposure day the animals were housed, 5 to a cage, in suspended stainless steel cages fitted with wiremesh floor and front in an animal room kept at a temperature of 22 ± 1°C and a relative humidity of 40-60 %. A 12 h light/12 h dark cycle was maintained.

Neither food nor water was present in the inhalation cylinders during the experimental periods. During the non-exposure periods the animals were provided with bottled tap water and the Institute's stock diet for rats, the composition of which is given in annex 1. Actual levels of contaminants in stock diet (batches prepared in the period September-December, 1985) and in

drinking water (determined in the same period) together with their tentative maxima are given in the annexes 2 and 3.

2.5 Generation of the test atmosphere

The formaldehyde gas was generated from paraformaldehyde by thermal depolymerization according to a method described by Chang et al. (1981).

2.6 Analysis of the test atmosphere

The cylinder atmospheres were monitored by two sytems. An automatic sampling and analyzing system, manufactured by SKALAR Analytical B.V. Breda, the Netherlands, was used to monitor the test atmospheres for continuous exposure (1 and 2 ppm). The method is based on a colorimetric estimation of formaldehyde by means of the Hantzsch reaction (also called the acetyl-acetone method; Nash, 1953). The SKALAR autoanalyzer was calibrated with standard samples of formaldehyde. A MIRAN 1A infra-red analyzer was used to monitor the test atmospheres for intermittent exposure (2 and 4 ppm).

The settings of the infra-red analyzer were as follows:

	MIRAN-1A
wavelength	3.58 µm
path length	18.75 m
slit	0.5 mm

The MIRAN-1A analyzer was calibrated against the acetylacetone method, as follows. The formaldehyde concentration in the test atmosphere was determined by analyzing a sample, collected in a washing bottle, by the acetylacetone method on the calibrated SKALAR analyzer (using this apparatus as a spectrophotometer) and by reading the absorbance of the test atmosphere from the MIRAN-1A infra-red analyzer. This was repeated 5 to 6 times with different concentrations of formaldehyde in the atmosphere.

2.7 Experimental design

The concentrations of the test substance during this 13-week study were as follows:

Group	colour code	target concentration (ppm)	concentration x time (ppm.h)
A	white	0	0
B	blue	1 (continuously)	8
C	green	2 (continuously)	16
D	red	2 (in 30-minute intervals)	8
E	yellow	4 (in 30-minute intervals)	16

2.8 Exposure scheme

The rats of groups B and C were exposed to the test substance for 8 hours a day continuously on 5 days a week for a period of 13 weeks. The rats of groups D and E were placed in the inhalation cylinders for 8 h a day and exposed to the test substance for 8 periods of 30 min per day with a non-exposure interval of 30 min after each 30 min of exposure. This exposure schedule was repeated each day, 5 days a week for a period of 13 weeks. The exposure to formaldehyde was started on September 19, 1985 and terminated on December 19, 1985.

2.9 Observations, analyses and measurements

2.9.1 Clinical observations

The general health status of the rats was assessed by visual inspection twice a day during working days, viz. just before and just after the exposure. During the weekends the animals were examined only once a day.

2.9.2 Body weight

The body weight of each animal was recorded just before the start of the first exposure to the test substance and once every week thereafter.

2.9.3 Cell proliferation

Eigtheen hours after the third day of exposure to formaldehyde 5 rats of each group were given a single dose of [^{3}H]thymidine (74 kBq per gram body weight) by ip injection. Two hours later the rats were anaesthetized with Nembutal, exsanguinated by cannulating the abdominal aorta and decapitated. The head of each rat was skinned and the lower jaw was removed. The nasal cavity of each rat was flushed with a neutral aqueous phosphate-buffered 4% formaldehyde solution and fixed in the same solution. After decalcification of the nose in nitric acid and sodium sulphate, 6 standard samples were taken (designated as levels I to VI, see annex 4) and processed through paraffin wax. The samples were oriented in the cassettes in such a way that the posterior surface was sectioned. Sections of 5 µm thickness, obtained from samples taken at cross levels II and III, were processed for autoradiography using Kodak NTB-2 emulsion. After 4 weeks of exposure at -28°C the slides were developed with Kodak D19, stained with haematoxylin and eosin and embedded in DePex. To determine the rate of cell turnover in the respiratory epithelium of the nose, 5,000 cells (where possible) lining the nasal and maxillary turbinates, septum and lateral wall of level II were scored for silver grains over the nuclei, in each rat of each dose group. The percentage of labeled cells was obtained by dividing the number of cells with 4 or more silver grains over the nucleus by the total number of cells counted. Sections of level III were not scored in this study.

At the end of the 13-week exposure period (for practical reasons on day 89 and day 90), 10 rats of each group were similarly treated with [^{3}H]thymidine. Sections (at level II) of the noses of 5 rats per group were used for the determination of the cell turnover; the sections of the other rats were kept in reserve. Sections of the noses of all rats were used for histopathological examination as decribed in the next paragraph.

2.9.4 Pathology

At the end of the 13-week test period (on day 91) the remaining rats (15 per group) were anaesthetized by ether, exsanguinated by cannulating the abdominal aorta and examined grossly for pathological changes. The nose of each rat was fixed in a neutral aqueous phosphate-buffered 4% formaldehyde solution. The nose was decalcified using nitric acid and sodium sulphate. Six standard samples of the nose were taken (see annex 4), processed through paraffin wax, sectioned at 5 µm, stained with haematoxylin and eosin, and examined microscopically.

2.10 Statistical analysis

Statistical analysis of body weights was carried out using analysis of covariance followed by the Dunnett test. For histopathological changes, Fisher's exact probability test was used. The data of the cell proliferation study were evaluated by Student's t-test.

2.11 Contributors

Major contributions to this study were made by :

Mr F. Hendriksma	: animal handling
Mr W.M. van Doorn	: animal handling
Mr J.W. Viljeer	: senior technician
Ms W.H. Stenhuis	: histotechnician
Mr T.G.J. Dirksen	: histotechnician
Mr W.R. Leeman	: cell proliferation study
Drs L.M. Appelman	: study director
Dr J.W.G.M. Wilmer	: deputy study director
Dr R.A. Woutersen	: pathologist
Dr V.J. Feron	: study supervisor

2.12 Deviations from the protocol

Animals were not exposed on day 19, due to technical problems.
The results of other subchronic inhalation toxicity studies with relatively low levels of formaldehyde, recently carried out in our Institute (Woutersen et al., 1984; Zwart et al. 1986), indicated that level II (see annex 4) of the nose is more appropriate than level III for studying the cytotoxic effects of formaldehyde on the rat nasal respiratory epithelium.
Therefore, it was decided to prepare sections both at level II and III of 10 (instead of 5) rats per group and to process these sections for autoradiography. Furthermore, it was decided to determine the rate of cell turnover first at level II (instead of at level III as indicated in the protocol), using sections of 5 rats per group.

3. RESULTS

3.1 Exposure levels

The actual mean concentrations of formaldehyde to which the rats in the different groups were exposed during the entire exposure period, were:

Group	target concentration (ppm)	actual concentration[1] (ppm)
A	0	0
B	1 (continuously)	0.99 ± 0.03
C	2 (continuously)	2.00 ± 0.06
D	2 (in 30-minute intervals)	2.00 ± 0.07
E	4 (in 30-minute intervals)	3.98 ± 0.10

[1] mean ± standard deviation

The actual mean daily concentrations of formaldehyde to which the rats in the different groups were exposed are listed in table 1. Generally, within

1 to 2 minutes after the start of an exposure the desired concentration of formaldehyde was reached. After termination of an exposure the concentration dropped to zero within 2 to 3 minutes.

3.2 Clinical observations

The behaviour and appearance of the animals were not visibly influenced by the exposure to formaldehyde.

3.3 Body weights (table 2)

Body weights of animals of the test groups did not show statistically significant differences with those of the controls during the entire exposure period.

3.4 Cell proliferation (table 3, annexes 5, 6 and 7)

The cell turnover rate in each group showed an approximately log-gaussian distribution. Statistics have been applied accordingly (annex 7). After 3 days of exposure, the number of [^{3}H]thymidine-labeled nasal respiratory epithelial cells (at level II) was reduced to about 50% of that of the controls, in the groups continuously exposed to 1 ppm or interruptedly to 2 ppm (receiving the same daily dose of 8 ppm.h). However, in both groups the difference with the controls was not statistically significant. Moreover, this phenomenon was not seen after 13 weeks of exposure. No statistically significant difference was observed in the number of labeled cells in any of the other test groups as compared to the controls.
At the end of the 13-week exposure period a slight increase in cell turnover rate was observed in the group interruptedly exposed to 4 ppm formaldehyde. The difference with the controls was not statistically significant. In all other test groups the cell turnover rate was comparable to that of the controls.

3.5 Pathology

3.5.1 Gross examination

Gross examination at autopsy did not reveal any abnormality that could be related to the inhalation of the test substance.

3.5.2 Microscopic examination (table 4)

Treatment-related histopathological changes were only found in the nose of animals interruptedly exposed to 4 ppm formaldehyde (total dose 16 ppm.h) and comprised:

a) an increased degree and incidence of disarranged respiratory epithelium lining the septum and nasoturbinates, and
b) an increased incidence of squamous metaplasia of the respiratory epithelium frequently accompanied by basal cell hyperplasia and, in 3 out of 25 animals, by keratinisation.

No increased incidences of these lesions were found in the 2 ppm group receiving the same daily dose of formaldehyde (16 ppm.h) as the 4 ppm group but obtained by means of daily 8-hour uninterrupted exposures to 2 ppm, sugggesting that in the 4 ppm group the concentration rather than the dose was responsible for the effects observed.

Increased incidences of epithelial disarrangement, nest-like infolds and goblet cell hyperplasia were seen in rats interruptedly exposed to 2 ppm formaldehyde (table 4). However, these lesions are <u>not</u> ascribed to formaldehyde but to the high incidence of rhinitis observed in this group, because rhinitis is usually accompanied by disarrangement and hyperplasia of the lining epithelium. Moreover, an increased incidence of the aforementioned lesions is not present in the group continuously exposed to 2 ppm formaldehyde.

The unusually high incidence of rhinitis in the group interruptedly exposed to 2 ppm is not considered a compound-related effect because:

a) the incidence of rhinitis in the group interruptedly exposed to 4 ppm formaldehyde was considerably lower than in the group interruptedly exposed to 2 ppm,
b) the incidence of rhinitis in the group continuously exposed to 2 ppm formaldehyde is considerably lower than in the group interruptedly exposed to 2 ppm, and
c) large variations between groups of rats in incidences of this nasal lesion are not uncommon in rats of this strain and age, which might be related to variation in the composition of the microbiological atmosphere in the different exposure cylinders.

4. DISCUSSION

From the results of the histopathological observations it appeared that cytotoxic effects of formaldehyde on the nasal respiratory epithelium only occurred after interrupted exposure of rats (8 successive 1 h periods a day, each consisting of 30 min of exposure and 30 min of non-exposure) to 4 ppm formaldehyde, on 5 days a week for 13 weeks. The lesions observed in the 4 ppm group mainly consisted of increased incidences of disarrangement of epithelium and squamous metaplasia occasionally accompanied by keratinisation. No lesions of the nasal epithelium regarded as compound-related were observed in any of the other test groups. The unusually high incidence of rhinitis found in the group interruptedly exposed to 2 ppm formaldehyde was considered to be a chance-effect unrelated to formaldehyde, and moreover, was held responsible for the high incidence of epithelial disarrangement and hyperplasia in this group.

The results of the cell proliferation study were in good agreement with the histopathological findings in the nose: after 13 weeks the cell turnover rate at level II of the nasal respiratory epithelium was almost 3 times higher in the 4 ppm group than in controls, whereas cell turnover rate in the other test groups was comparable to that in controls. Surprisingly, we did not find any increase in cell turnover rate in the 4 ppm group after 3 days of exposure. This result is highly unexpected, because previous investigations showed a marked increase in the cell turnover rate after 3

days of exposure to 3, 5 or 6 ppm formaldehyde (Zwart et al., 1986; Wilmer et al., 1985; Swenberg et al., 1983). These studies also showed that the increase in cell turnover rate decreased with time despite continuation of the exposure to formaldehyde, which renders the absence of an increased cell turnover in the 4 ppm group after 3 days in the present study more puzzling, particularly in view of the (still) almost 3 times higher cell turnover rate in this group after 13 weeks. The results of the study by Zwart et al. (1986) showed a marked increase in cell turnover as compared to controls both at level II and at level III, after 3 days of exposure to 3 ppm formaldehyde. After 13 weeks of exposure a significant increase in cell turnover rate was seen only at level II. The cell turnover rate was determined only in cells lining the septum, which appeared to be (for unknown reasons) so unexpectedly low in the controls that the significance of the findings obtained in rats exposed to low levels of formaldehyde (up to 3 ppm) might be arguable. In view of the results obtained at level II in that study, however, the cell turnover rate in the present study was determined only at that level II. The number of labeled cells was scored in 5000 cells per rat, distributed over the respiratory epithelium of the nasal turbinates (1000 cells), maxillary turbinates (1000 cells) and the septum (3000 cells). Although the cell turnover rate of controls was more comparable to that of previous studies, no sound explanation can be given for the unexpected findings in the 4 ppm group after 3 days of exposure.

Unlike the animals interruptedly exposed to 4 ppm formaldehyde, the rats of the 2 ppm group receiving the same daily dose (16 ppm.h) as the animals of the 4 ppm group, did not show compound-related nasal lesions. This points to the concentration rather than the dose of formaldehyde being primarily responsible for the effects on the nasal epithelium. The same conclusion was drawn from the results of a previous formaldehyde study in rats of similar design and conduct but using higher, clearly cytotoxic exposure levels (5 up to 20 ppm) and a much shorter treatment period (4 weeks) (Wilmer et al., 1985 and 1986). Also Swenberg et al. (1983) came to the same conclusion in their studies in rats with single exposures of 3 to 12 hours (one day treatment) and exposure concentrations of 3 to 12 ppm. Apparently, the conclusion that the concentration is more important than the dose for its cytotoxic effect on the nose holds for exposure levels ranging from

marginally to severely cytotoxic and for exposure periods varying from a few hours to several months.

In the present study 2 ppm formaldehyde was found to be a non-toxic effect level for the nasal epithelium. This finding is in full agreement with the results of previous inhalation studies in which rats were exposed to formaldehyde at levels varying from 0.2 to 20 ppm, for 6 to 22 hours a day during 13 or 26 weeks (Rush et al., 1983; Woutersen et al., 1984; Zwart et al., 1986). In these studies formaldehyde was found to damage the nasal mucosa at levels of 3 ppm and higher; at levels of 1 ppm and lower nasal lesions unequivocally attributable to formaldehyde were not found. On the other hand, 2 ppm formaldehyde has been found to induce squamous metaplasia of the nasal respiratory epithelium after exposure periods of 12 months and longer (Swenberg et al., 1983; Kerns et al., 1983). This positive finding with 2 ppm formaldehyde after 12 months and longer, in combination with our negative result with 2 ppm after 13 weeks, may indicate that in the long run the cytotoxicity of formaldehyde for the nasal mucosa is more dependent on the dose than on the concentration. However, another explanation could be that the nasal epithelium of older rats is more sensitive to the cytotoxic action of formaldehyde than that of younger rats.

With respect to the "Maximum Allowable Concentration" of formaldehyde, which has been set in the Netherlands at 1 ppm with a ceiling of 2 ppm (to be taken as 0.5 h time weighted average of 8 ppm.h), it seems justifiable to conclude that the results of the present study do not give rise to change the MAC value:

a) a ceiling of 2 ppm formaldehyde (to be taken as 0.5 h time weighted average of 8 ppm.h) is highly desirable because this study again demonstrated the importance of the exposure concentration for the cytotoxicity of formaldehyde, and
b) the study again underlined that 1 ppm formaldehyde is low enough to avoid cytotoxic effects on the nasal epithelium. This is of particular importance since in the case of formaldehyde avoiding nasal damage means reducing the risk of nasal cancer to virtually nought (Feron et al., 1984).

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CIVO/TNO
STUDY NO 803 SUB-CHRONIC (13 WEEK) INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN RAT

TABLE I . MEAN DAILY CONCENTRATION IN THE AIRFLOW (PPM).

M A L E S

	1 PPM MEAN	2 PPM MEAN	2 PPM* MEAN	4 PPM* MEAN
DAY 0	0.98	2.00	1.99	4.01
DAY 1	1.01	2.01	1.96	4.01
DAY 4	0.96	2.00	1.99	4.02
DAY 5	1.01	2.01	2.01	3.95
DAY 6	1.00	2.00	2.02	4.04
DAY 7	1.00	2.00	2.03	3.99
DAY 8	1.00	2.00	2.01	4.02
DAY 11	1.00	1.99	2.03	4.00
DAY 12	1.00	2.00	2.03	4.00
DAY 13	0.99	2.00	1.98	4.01
DAY 14	1.00	2.00	2.06	3.97
DAY 15	1.00	2.00	2.02	4.04
DAY 18	0.96	1.94	2.02	3.96
DAY 19	--	--	--	--
DAY 20	0.99	1.98	1.96	3.94
DAY 21	1.01	2.03	2.03	4.00
DAY 22	0.99	1.99	1.99	3.90
DAY 25	0.95	1.93	1.98	3.93
DAY 26	1.02	2.00	2.03	3.75
DAY 27	1.04	1.99	2.05	3.91
DAY 28	1.04	2.03	2.04	3.96
DAY 29	1.00	2.00	2.01	3.96
DAY 32	1.00	1.88	1.94	3.96
DAY 33	0.96	2.12	2.01	3.81
DAY 34	1.03	2.03	2.09	3.97
DAY 35	1.04	2.03	2.08	3.95
DAY 36	0.97	1.96	1.93	3.83
DAY 39	0.95	1.93	1.94	3.77
DAY 40	0.96	1.88	2.02	3.97

* = INTERRUPTED EXPOSURE

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STUDY NO 803 SUB-CHRONIC (13 WEEK) INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN RATS

TABLE I . MEAN DAILY CONCENTRATION IN THE AIRFLOW (PPM).

M A L E S

	1 PPM MEAN	2 PPM MEAN	2 PPM* MEAN	4 PPM* MEAN
DAY 41	1.02	1.99	1.90	4.12
DAY 42	0.99	1.94	1.93	4.16
DAY 43	1.01	1.97	1.94	3.92
DAY 46	1.01	1.98	1.77	4.00
DAY 47	0.99	2.12	2.08	4.28
DAY 48	1.00	2.01	1.95	4.03
DAY 49	1.00	2.04	2.09	4.15
DAY 50	0.98	1.96	2.06	4.06
DAY 53	0.96	1.98	1.97	3.90
DAY 54	0.98	2.06	2.07	4.15
DAY 55	1.02	2.06	2.07	4.05
DAY 56	0.99	2.01	2.10	3.91
DAY 57	1.00	2.05	2.07	4.01
DAY 60	0.96	2.05	2.02	3.96
DAY 61	1.00	1.99	2.03	4.07
DAY 62	0.99	1.97	1.87	3.83
DAY 63	0.98	2.00	1.97	4.01
DAY 64	1.04	2.01	2.06	4.01
DAY 67	1.02	2.00	1.99	3.90
DAY 68	0.95	2.00	2.02	4.02
DAY 69	0.99	2.25	2.17	4.32
DAY 70	0.98	1.95	1.99	3.93
DAY 71	0.92	1.91	1.95	3.97
DAY 74	0.94	1.95	1.89	4.04
DAY 75	0.89	2.01	1.72	3.84
DAY 76	1.00	2.06	2.02	3.94
DAY 77	0.96	1.94	1.98	3.84
DAY 78	1.02	2.02	2.04	4.03
DAY 81	1.01	1.80	1.86	3.81
DAY 82	0.98	2.07	2.09	4.11

* = INTERRUPTED EXPOSURE

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STUDY NO 803 SUB-CHRONIC (13 WEEK) INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN RATS

TABLE I . MEAN DAILY CONCENTRATION IN THE AIRFLOW (PPM).

M A L E S

	1 PPM MEAN	*	2 PPM MEAN	2 PPM* MEAN	4 PPM* MEAN
DAY 83	1.03		1.98	2.02	4.09
DAY 84	0.97		2.04	2.02	3.98
DAY 85	0.97		2.01	2.01	3.99
DAY 88	0.93		1.97	1.91	3.92
DAY 89	0.95		1.97	2.04	4.01
DAY 90	0.91		1.97	1.95	3.88
GRAND MEANS:	0.99		2.00	2.00	3.98

* = INTERRUPTED EXPOSURE

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STUDY NO 802 SUB-CHRONIC (13 WEEK) INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN RATS ID.NO = 3432

TABLE 2 . MEAN BODY WEIGHTS (G).

M A L E S

	CONTROL			1 PPM			2 PPM			2 PPM*			4 PPM*		
	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
DAY 0	58.3	1.0	25	58.7	1.1	25	58.4	0.9	25	59.6	0.8	25	59.9	1.0	25
DAY 7	93.6	1.4	25	94.4	1.3	25	93.5	1.2	25	95.2	1.3	25	96.7	1.6	25
DAY 14	130.8	2.1	25	131.9	1.8	25	130.4	1.6	25	130.0	2.0	25	130.1	2.1	25
DAY 21	169.9	2.6	25	171.3	2.6	25	169.1	2.2	25	168.5	3.1	25	163.9	3.7	25
DAY 28	207.7	3.4	25	213.5	2.9	25	209.9	2.7	25	202.6	3.9	25	199.8	4.7	25
DAY 35	245.5	4.2	25	249.0	3.1	25	245.4	2.9	25	241.7	4.3	25	240.2	4.2	25
DAY 42	271.3	4.3	25	271.6	3.7	25	271.7	3.3	25	266.7	4.7	25	266.5	4.5	25
DAY 49	300.4	4.6	25	300.8	3.8	25	298.0	3.8	25	292.6	5.2	25	293.4	4.6	25
DAY 56	320.7	4.8	25	321.5	4.1	25	319.9	4.3	25	312.6	5.5	25	314.2	4.8	25
DAY 63	334.8	5.2	25	339.4	4.5	25	336.5	5.0	25	329.6	5.4	25	334.3	4.9	25
DAY 70	345.3	5.4	25	349.4	4.9	25	347.1	5.1	25	339.9	5.1	25	344.0	5.1	25
DAY 77	353.3	5.8	25	357.4	5.4	25	353.6	5.1	25	348.6	5.3	25	352.1	5.2	25
DAY 84	360.4	6.0	25	360.4	5.3	25	361.9	5.0	25	358.3	5.5	25	360.8	5.5	25
DAY 89	355.9	21.1	5	366.1	13.4	5	358.8	10.4	5	375.3	9.3	5	344.2	12.8	5
DAY 90	381.3	6.7	5	381.5	9.2	5	376.4	12.8	5	367.1	16.6	5	376.7	16.8	5
DAY 91	367.9	6.9	15	372.0	8.0	15	367.7	6.3	15	363.1	7.5	15	373.1	5.8	15

STATISTICS: COVAR + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)

TABLE 3: SUBCHRONIC INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN RATS
SUMMARY OF THE RESULTS OF THE CELL PROLIFERATION STUDY

Group[1]	formaldehyde concentration (ppm)	formaldehyde concentration x time (ppm.h)	% labeled cells	
			after 3 days of exposure	after 13 weeks of exposure
A	0	0	0.60 ± 0.37	1.03 ± 0.26
B	1	8	0.34 ± 0.10	0.81 ± 0.54
C	2	16	0.61 ± 0.28	0.91 ± 0.59
D	2	8	0.29 ± 0.20	1.16 ± 0.59
E	4	16	0.58 ± 0.32	2.86 ± 1.80

[1] A = fresh air only; B = 1 ppm, 8 hours continuously;
C = 2 ppm, 8 hours continuously; D = 2 ppm in intervals;
E = 4 ppm in intervals.

TABLE 4: SUBCHRONIC INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN RATS. SUMMARY OF HISTOPATHOLOGICAL CHANGES IN THE NOSE AFTER 13 WEEKS[1]

Site and type of changes observed[2]	Incidence of lesions MALES				
	A[3]	B	C	D	E
NOSE	(25)	(22)	(24)	(23)	(25)
Respiratory epithelium at level II					
1. Disarrangement					
- focal	12	4	8	3*	8
- diffuse	1	1	0	15***	11**
2. Necrosis					
- focal	4	3	0	2	3
- diffuse	0	0	0	2	2
3. Basal cell hyperplasia					
- focal	9	4	6	11	10
- diffuse	4	0	0	4	11
4. Squamous metaplasia					
- focal	5	0	1	7	16**
5. Keratinisation	0	0	1	0	3
6. Nest-like infolds					
- focal	5	4	11	14**	7
- diffuse	0	3	1	0	1

(continued on page 28)

TABLE 4: SUBCHRONIC INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN RATS. SUMMARY OF HISTOPATHOLOGICAL CHANGES IN THE NOSE AFTER 13 WEEKS[1] (CONTINUED)

Site and type of changes observed[2]	Incidence of lesions MALES				
	A[3]	B	C	D	E
NOSE	(25)	(22)	(24)	(23)	(25)
Respiratory epithelium at level II					
7. Goblet cell hyperplasia					
- focal	0	1	1	2	1
- diffuse	5	2	8	13*	10
8. Rhinitis	3	2	3	16***	8

[1] The number of animals examined is given in brackets
[2] Lesions most probably related to the treatment
[3] A = fresh air only; B = 1 ppm, 8 hours continuously; C = 2 ppm, 8 hours continuously; D = 2 ppm in intervals; E = 4 ppm in intervals.

ANNEX 1 : COMPOSITION OF THE INSTITUTE'S STOCK DIET FOR RATS

soya bean oil meal	11
fish meal	7
meat and bone scraps	4
wheat (whole ground)	36
maize (whole ground)	29.7
brewer's yeast	3
grass meal	3
whey powder	2
defatted bone meal	0.4
salt with trace elements [1]	0.5
B-vitamin mixture [2]	0.1
vitamin ADEK mixture [3]	0.3
soya bean oil	3
	100.0

[1] Trace elements in salt

	Batch		added to 1 kg diet	
$MnSO_4.H_2O$	2380	g	41	mg Mn
$ZnCl_2$	250	g	12	mg Zn
KJ	20	g	1.5	mg J
$FeSO_4.7H_2O$	1250	g	25	mg Fe
$CoCl_2.6H_2O$	20	g	0.7	mg Co
$CuSO_4.5H_2O$	400	g	8	mg Cu
NaCl	45680	g		
	50000	g		

[2] B-vitamin mixture

	Batch		added to 1 kg diet	
Thiamin-HCl	25	g	2.5	mg
Riboflavin	30	g	3.0	mg
Pyridoxin-HCl	100	g	10.0	mg
Niacin	125	g	12.5	mg
Ca-pantothenate	75	g	7.5	mg
Biotin	1.5	g	0.15	mg
Folic acid	5	g	0.5	mg
Vitamin B_{12}-prep. (0.1 %)	50	g	5.0	mg (= 5 µg B_{12})
Ground sucrose	9588.5	g		
	10000	g		

[3] Vitamin ADEK mixture

	Batch		added to 1 kg diet	
Vit. AD_3-prills (Farmix): 2250 IU vit. A, 750 IU vit. D_3/g	25000	g	6339 IU vit. A 2112 IU vit. D_3	
Vit. E-dry powder (Merck) 50 %	800	g	45	mg vit. E
Menadion-Na-bisulphite (Vit. K_3)	27	g	3	mg vit. K_3
Wheat starch	800	g		
	26627	g		

ANNEX 2 : CONTAMINANTS IN THE STOCK DIET
(5000 kg batch, produced by Van Eck, Cothen, on August 16, 1985)

lead		0.65	mg/kg
cadmium		0.06	mg/kg
mercury		0.015	mg/kg
tin	<	1	mg/kg
arsenic		0.5	mg/kg
selenium		0.5	mg/kg
fluorine		10	mg/kg
organo-P-compounds		ND	
- malathion		0.12	mg/kg
- pirimifos-methyl		0.05	mg/kg
organo-Cl-compounds		ND	
PCB's		ND	
dithiocarbamates	<	0.5	mg/kg
aflatoxin B_1, B_2, G_1, G_2	<	2	µg/kg
oestrogenic activity (Tiecco test)		ND	
benzo(a)pyrene		1.4	µg/kg
benzo(b)fluoranthene		1.7	µg/kg
indeno(1,2,3-c,d)pyrene	<	2.5	µg/kg
benzo(k)fluoranthene		0.7	µg/kg
fluoranthene		6.4	µg/kg
benz(ghi)perylene		2.0	µg/kg
K-nitrate		60	mg/kg
Na-nitrite		4	mg/kg
nitrosodimethylamine		2.0	µg/kg
nitrosopyrrolidine		2.4	µg/kg
other nitrosamines		ND	µg/kg
urease activity		0.03	Δ pH
trypsin inhibitor	<	1.0	mg tryps/g sample
haemagglutinins	positive dilution	none	
	negative dilution	1:10	

ND = not detectable

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ANNEX 3 : CONTAMINANTS IN THE DRINKING WATER (determined in period Aug.-Dec. 1985)

	ppb		ppm
lead	< 1	ammonium	< 0.01
cadmium	< 1	nitrate	8.7
mercury	< 5	nitrite	< 0.01
arsenic	< 1	chloride	24
selenium	< 5	sodium	15.0
copper	30	calcium	28.0
chromium	< 1	potassium	1.2
zinc	< 10	iron	0.05
cyanide	< 5		

	ppb		ppb
dichloromethane	< 2	HCB	< 0.01
1,2-dichloroethane	< 2	α-HCH	< 0.01
chloroform	< 0.1	lindane	< 0.01
1,1,1-trichloroethane	< 0.1	heptachlor	< 0.01
tetrachloromethane	< 0.05	heptachl. epoxide	< 0.02
trichloroethylene	0.3	aldrin	< 0.02
tetrachloroethylene	< 0.1	dieldrin	< 0.03
		α-chlordane	< 0.03
		γ-chlordane	< 0.03
		endrin	< 0.05
		p,p'-DDE	< 0.03
		o,p'-DDT	< 0.06
		p,p'-TDE	< 0.06
		p,p'-DDT	< 0.1
		ß-HCH	< 0.03
		methoxychlor	< 0.15
		PCB's	< 0.8
		DDVP	< 0.1
		mevinfos	< 0.1
		diazinon	< 0.1
		malathion	< 0.2
		parathion	< 0.2
		chlorpyrifos	< 0.1
		sulfotep	< 0.1
		fenitrothion	< 0.2

	ppt
benzo(a)pyrene	< 5
benzo(b)fluoranthene	< 5
indeno(1,2,3,-c,d)pyrene	< 10
benzo(k)fluoranthene	< 5
fluoranthene	< 20
benzo(g,h,i)perylene	< 10

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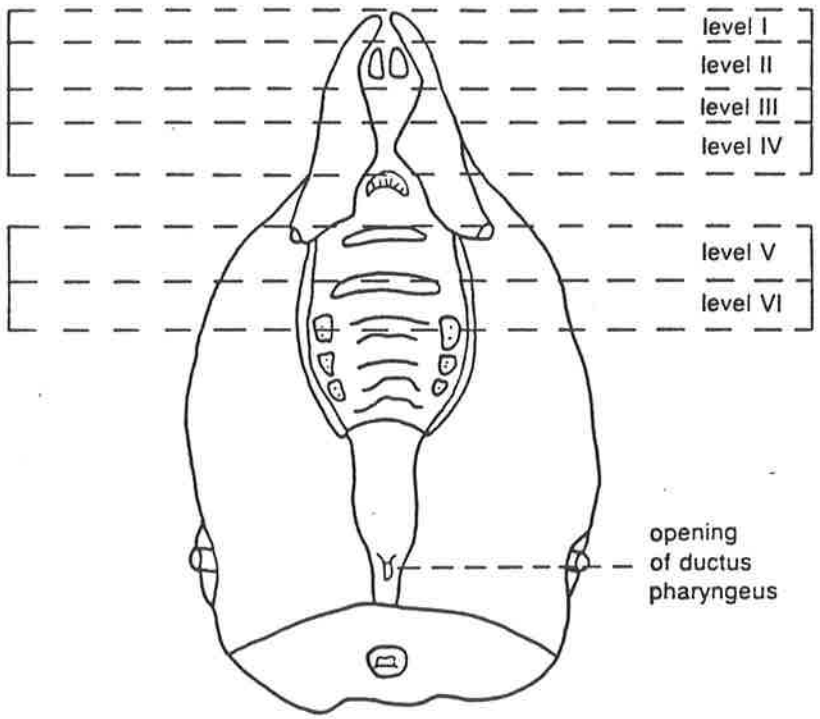
ANNEX 4 : DIAGRAM OF CROSS LEVELS OF THE NOSE USED FOR SECTIONING



ANNEX 5: SUBCHRONIC INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN MALE RATS. RESULTS OF THE CELL PROLIFERATION STUDY AFTER 3 DAYS: INDIVIDUAL DATA (LEVEL II)

Group[1]	Slide 1		Slide 2		Total		% Labeled cells
	n.l.	l.	n.l.	l.	n.l.	l.	
A2	2483	17	2490	10	4973	27	0.54
A4	2482	18	2481	19	4763	37	0.74
A6	2474	26	2468	32	4942	58	1.16
A8	2491	9	2491	9	4982	18	0.36
A10	2493	7	2496	4	4989	11	0.22
A(total)					24849	151	0.60 ± 0.37
B2			no labeled cells				
B4	2493	7	2485	15	4978	22	0.44
B6	2490	10	2496	4	4986	14	0.28
B8	2493	7	2487	13	4980	20	0.40
B10	2493	7	2495	5	4988	12	0.24
B(total)					19932	68	0.34 ± 0.10
C2	2479	21	2474	26	4953	47	0.94
C4	2477	23	2479	21	4956	44	0.88
C6	2489	11	2494	6	4983	17	0.34
C8	2489	11	2487	13	4976	24	0.48
C10	2491	9	2489	11	4980	20	0.40
C(total)					24148	152	0.61 ± 0.28
D2	2487	13	2482	18	4969	31	0.62
D4	2497	3	2498	2	4995	5	0.10
D6	2494	6	2494	6	4988	12	0.24
D8	2490	10	2496	4	4986	14	0.28
D10	2495	5	2495	5	4990	10	0.20
D(total)					24928	72	0.29 ± 0.20
E2	2477	23	2469	31	4946	54	1.08
E4	2485	15	2490	10	4975	25	0.50
E6	2491	9	2487	13	4978	22	0.44
E8	2495	5	2494	6	4989	11	0.22
E10	2482	18	2486	14	4968	32	0.64
E(total)					24856	144	0.58 ± 0.32

[1] Data are given separately for each animal and per dose group. Per cross level, two 5 µm sections were used to score the cells.
[2] n.l. = non labeled cells
l. = labeled cells

ANNEX 6: SUBCHRONIC INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN MALE RATS. RESULTS OF THE CELL PROLIFERATION STUDY AFTER 13 WEEKS: INDIVIDUAL DATA (LEVEL II)

Group[1]	Slide 1		Slide 2		Total		% Labeled cells (± SD)
	n.l.	l.	n.l.	l.	n.l.	l.	
A2	2486	14	2468	32	4954	46	0.92
A12	2469	31	2478	22	4945	53	1.06
A14	2482	18	2485	15	4967	33	0.66
A24	2468	34	2468	34	4932	68	1.36
A42	2469	31	2474	26	4943	57	1.14
A(total)					24743	257	1.03 ± 0.26
B2	2473	27	2471	29	4944	56	1.12
B14	2493	7	2489	11	4982	18	0.36
B22	2461	39	2478	22	4939	61	1.22
B32	2468	32	2469	31	4937	63	1.26
B44	2496	4	2499	1	4995	5	0.10
B(total)					24797	203	0.81 ± 0.54
C4	2477	23	2495	5	4972	28	0.56
C12	2492	8	2488	12	4980	20	0.40
C24	2492	8	2477	23	4969	31	0.62
C34	2460	40	2486	16	4944	56	1.12
C42	2436	64	2471	29	4907	93	1.86
C(total)					24772	228	0.91 ± 0.59
D2	2489	11	2486	14	4975	25	0.50
D22	2491	9	2466	34	4957	43	0.86
D32	2446	54	2489	11	4935	65	1.30
D34	2474	26	2473	27	4947	53	1.06
D44	2478	22	2418	82	4896	104	2.08
D(total)					24710	290	1.16 ± 0.59
E2	2437	63	2451	49	4888	112	2.24
E12	2468	32	2470	30	4938	62	1.24
E22	2371	129	2453	47	4824	176	3.52
E32	2385	115	2331	169	4716	284	5.68
E42	2454	46	2465	35	4919	81	1.62
E(total)					24285	715	2.86 ± 1.80

[1] Data are given separately for each animal and per dose group. Per cross level two 5 µm sections were used to score the cells.

[2] n.l. = non labeled cells
l. = labeled cells

ANNEX 7: SUBCHRONIC INHALATION TOXICITY STUDY WITH FORMALDEHYDE IN MALE RATS. RESULTS OF THE LOG-GAUSSIAN STATISTICS OF THE CELL PROLIFERATION STUDY.

Group[1]	formaldehyde concentration (ppm)	formaldehyde concentration x time (ppmh)	log cell turnover (%) after 3 days of exposure	log cell turnover (%) after 13 weeks of exposure
A	0	0	- 0.29 ± 0.28	0.00 ± 0.12
B	1	8	- 0.48 ± 0.12	- 0.24 ± 0.48
C	2	16	- 0.25 ± 0.20	- 0.11 ± 0.27
D	2	8	- 0.62 ± 0.29	0.02 ± 0.23
E	4	16	- 0.30 ± 0.25	0.39 ± 0.26

[1] A = fresh air only; B = 1 ppm, 8 hours continuously;
C = 2 ppm, 8 hours continuously; D = 2 ppm in intervals;
E = 4 ppm in intervals.