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Hyperplasia of the lymphoepithelium of NALT in rats but not in mice upon 28-day exposure to 15 ppm formaldehyde vapor

C. Frieke Kuper^a, Lidy van Oostrum^a, Lan Ma-Hock^b, Stefan Durrer^b, Ruud A. Woutersen^a

^a Department of Toxicology and Applied Pharmacology, TNO Quality of Life, 3704HE, Zeist, the Netherlands

^b BASF SE, Department of Product Safety, 67056 Ludwigshafen, Germany

Corresponding author: C. Frieke Kuper, Department of Toxicology and Applied Pharmacology, TNO Quality of Life, 3704HE, Zeist, the Netherlands, frieke.kuper@tno.nl; Tel. +31 30 6944 478

Abbreviations:, FA = formaldehyde, BrdU = bromodeoxyureidine, F = follicular, HCl = hydrogen chloride, H&E = hematoxylin and eosin, IF = interfollicular, LLNA = Local lymph node assay, NALT = nasopharynx-associated lymphoid tissue, PBS = phosphate-buffered saline, PCA = principal component analysis, PCDA = principal component discriminant analysis, SD = standard deviation, STP = Society of Toxicologic Pathologists

Abstract

To investigate if local lymphoid tissues are a target of FA, nasopharynx-associated lymphoid tissues (NALT) and upper-respiratory tract-draining lymph nodes were examined in a 28-day inhalation study with FA vapor in Fischer-344 rats and B6C3F1 mice.

Paraffin-embedded tissues were sectioned and stained with H&E or stained immunohistochemically for cell proliferation (BrdU incorporation).

Light microscopy revealed simple hyperplasia of NALT lymphoepithelium of rats exposed to 15 ppm and an increased proliferation rate of the epithelial cells. Principal component (discriminant) analysis of rat NALT and lymph nodes data did not reveal other effects or effects at lower exposure levels. Mice tissues were not affected.

It was concluded that hyperplasia of the lymphoepithelium of NALT of rats exposed to 15 ppm was the only distinct effect of FA vapor on local lymphoid tissues (NALT and lymph nodes) of Fischer-344 rats and B3C3F1 mice.

Key words: Cervical lymph nodes; BrdU; lymphoepithelium; hyperplasia; PCA

Introduction

Formaldehyde (FA) is a chemical with a high annual production and widespread use, and thus many people are exposed to it. Studies in humans have shown that exposure to FA by inhalation is associated with respiratory symptoms, and eye, nose and throat irritation. In addition, FA is considered by the IARC as a human carcinogen, related to nasopharyngeal cancers (IARC Monographs, 2006). Animal studies have supported the relationship between FA exposure and local toxicity, including epithelial tumors/carcinomas (Kerns et al., 1983). The rat was the most sensitive species to nasal cancer induction by FA.

IARC also concluded that there was strong but not sufficient evidence of a causal association between hematological tumors, especially myeloid leukemia, and occupational exposure to FA. This was based on 2 of 3 large industrial cohort studies and a large meta-analysis of all available epidemiological data. The results of studies in animals with respect to hematopoietic tumors are under considerable debate. A relationship was suggested between FA-exposed female B6C3F1 mice and lymphomas by Devoney et al. (2006), based on lymphoma incidences of interim- and terminal-sacrificed animals combined.

The lack of a plausible mode of action lead IARC to conclude that based on the data available, it was not possible to identify a mechanism for the induction of myeloid leukemia and other hematological tumors in humans by FA and it was stated that this needed more research. Most chemically induced human leukemias are acute myeloid leukemias and precursor myelodysplastic syndromes. Almost all leukemias originate in the pluripotent stem cells and progenitor cells, which are mainly located in the bone marrow but they circulate also in low numbers in the peripheral blood. Established chemical leukemogens are genotoxic and are capable of inducing toxicity to the blood forming system. FA is

genotoxic but data on FA hematotoxicity are limited and inconsistent (reviewed by Zhang et al. 2009; Pyatt et al., 2008).

Another potential mechanism by which FA may have induced hematological tumors was proposed by Devoney et al. (2006). They acknowledge the reactivity and local toxicity of FA and propose that local lymphoid tissues are the target for malignant transformation by FA. Inhaled FA may act upon nasopharynx-associated lymphoid tissue (NALT), single mucosal lymphocytes or local lymph nodes. This mode of action would mainly explain part of the spectrum of hematological tumors, namely the lymphoproliferative disorders. Myeloid leukemias need malignant transformation of pluripotent stem cells, which might be present in NALT or nasal mucosa.

Fischer rats and B6C3F1 mice were exposed for 28 days to FA vapor at a range of concentrations, from 0.5 ppm (0.6 mg/m³) to 15 ppm (19 mg/m³). NALT and upper-respiratory tract-draining lymph nodes were sampled for microscopic examination. The lymph nodes were referred to as the superficial cervical lymph nodes (Tilney, 1971; *synonym*: mandibular lymph nodes, reviewed by Van den Broeck et al., 2006) and the posterior cervical lymph nodes (Tilney, 1971; *synonyms*: deep cervical, deep cranial lymph nodes, reviewed by Van den Broek et al., 2006). Paraffin-embedded sections of the lymphoid tissues were examined (semi)quantitatively for histopathological lesions and for changes in activation, according to the so-called enhanced histopathology approach (ICICIS, 1998; Kuper et al., 2000; Haley et al., 2005; Maronpot, 2006). In addition, cell proliferation was determined, based on immunohistochemical staining for BrdU.

Materials and Methods

Animals

Male Fischer rats (F-344/DuCrI) and female B6C3F1/CrI mice were obtained from Charles River Laboratories (Sulzfeld, Germany). They were housed under conventional conditions and were about 9 weeks old at the start of the exposure. Their weight at the start of the study was 206.0 ± 14.2 g for rat and 20.7 ± 1.2 g for mice. The in-life part of the study was performed at BASF SE (Ludwigshafen, Germany), an AAALAC-approved laboratory and in accordance with the German Animal Welfare Act and the European Council Directive 86/609/EEC, under good laboratory practice procedures.

Experimental conditions

The animals were exposed whole body to FA vapor (CAS 500-00-0, EU number, 10.21 % in water) for 6 hours per day, 5 days a week, during 4 weeks (Table 1). During exposure, the animals were housed singly in wire cages (DKIII for rats and DKI for mice) that were located in glass-steel inhalation chambers (1.4 m^3). Relative humidities in the inhalation chambers were measured with dielectric probes (Testo, Lenzkirch, Germany); temperatures were measured with thermoelements located in the exposure chambers; and air flow rates with differential pressure transmitters (Rosemount, Wessling, Germany). FA in the test atmosphere was monitored with an calibrated infrared spectrometer Gasmeter DX4000 (Ansyco, Karlsruhe, Germany) configured for formaldehyde.

BrdU-minipumps (ALZET osmotic minipumps, type 2001 for rats and 1003D for mice, Alzet Corporation, Palo Alto, USA) were implanted subcutaneously at the dorsal region, under isoflurane narcosis and an s.c. injection of buprenorphine for pain relieve. The implantation was performed 3 days before necropsy.

Tissue sampling

The posterior and superficial cervical lymph nodes and the heads, with skin and under jaw removed, were collected at BASF SE. The nasal tissues were first fixated by injection of the fixative via the nasopharyngeal opening in the palate and then the heads were immersed in the fixative. The lymph nodes were fixated by immersion. Tissues were fixated in Harrison's fixative (consisting of absolute ethanol, glacial acetic acid, 40% formaldehyde and saline; Harrison, 1984) for a maximum time period of 48 hours and subsequently transferred to 70% ethanol. Thereafter, they were shipped to TNO for further processing and microscopic examination under good laboratory practice regulations.

NALT was collected from the fixated heads as follows. First, the soft tissue of the palate was removed. The heads of the rats were then split longitudinally, beginning with an incision in the palate. In this way, the nasopharyngeal duct including NALT were exposed (Fig. 1). NALT was removed from the crevices and the surrounding tissue, using a 1 ml syringe with bended needle. NALT from mice was sampled without splitting the head, because it is situated more posteriorly in the nasal passages compared to rats (Fig. 2).

Histotechnical procedures

The lymph nodes and NALT were processed and embedded in paraffin wax and sectioned at 5 μm . NALT was embedded in such a way that the organ was sectioned longitudinally. Two sections per NALT were stained with hematoxylin and eosin (H&E). Two other sections were stained immunohistochemically with anti-BrdU (series 1 and 2). Of the lymph nodes, one section was stained with H&E and another was stained immuno-histochemically with anti-BrdU antibody.

For immunohistochemistry, the sections from the rat tissues and mouse NALT were stained, using a two-step indirect immunolabeling. Endogenous peroxide was blocked by 30 minutes incubation with 0.3% hydrogen peroxide. DNA was made single-stranded with 2N HCl for 30 minutes by 37 C. Antigen was then unmasked by incubation of the sections with 0.05% pronase in PBS for 10 minutes. Unspecific binding was then blocked with 25% normal goat serum in PBS for 15 minutes. The sections were incubated next with the monoclonal mouse anti-BrdU (Beckton and Dickinson, San Jose, California, USA, the Netherlands) for 60 minutes. Thereafter, they were incubated with Powervision (Labvision inc.; supplied by Klinipath, Duiven, the Netherlands) for 30 minutes, and stained with NovaRed (Vector; supplied by Brunswick, Amsterdam, the Netherlands). Finally, the sections were slightly counterstained with haematoxylin for 10 seconds.

The lymph nodes of mice were stained differently using a three-step labelling, because of false-positive (i.e. non-BrdU) staining of plasma cells by rat Powervision (Labvision inc.; supplied by Klinipath, Duiven, the Netherlands). The procedures for blocking of endogenous peroxidase, single-stranded preparation of DNA, unmasking of the antigen and blocking of the aspecific binding were identical as described above. Next, the sections were incubated with the monoclonal rat anti-BrdU (Abcam, Cambridge, UK) for 60 minutes, then incubated with rabbit-anti rat biotin (Dako, Glostrup, Denmark) for 30 minutes and incubated with streptavidine-horse radish peroxidase (Labvision Inc., supplied by ThermoScientific, California, USA) for 30 minutes. Lastly, they were stained as described above with NovaRed, followed by haematoxylin.

Light microscopy

The H&E-stained sections were examined microscopically, according to the approach of ICICIS (1998) and Kuper et al. (2000), and as described in STP Best practice (Haley et al., 2005). The size of NALT and the number of germinal centres in NALT and lymph nodes were chosen as parameters

indicative of activation. The size was determined by measuring the length and the broadest width of NALT on microphotographs of NALT, using the Axiocam microphotograph programme (Axiovision release 4.6.3; 04-2007) on a stereomicroscope (Zeiss, Sliedrecht, the Netherlands). Length and width were multiplied (surface area) and the result was translated into a degree (very small, small, medium, large and very large). Germinal centres were counted and the counts were translated into degrees (very slight, slight, moderate, marked/severe, very marked/very severe). The microscopic examination included also the 'conventional' histopathological examination, especially of the stroma and epithelium.

The BrdU-stained sections of lymph node and NALT were examined quantitatively, without previous knowledge of the treatment ('blind scoring'). The two series of NALT were counted on separate occasions. BrdU-positive cells in the lymphoepithelium were counted per 0.2 mm (0.195 mm, last digit estimated) epithelium, with the use of a grid that was divided into 100 units. The BrdU-positive cells in the other compartments were counted per 0.04 square mm, with the use of the same grid. In case the compartments were too small to cover the full grid, the number of uncovered units was counted and a correction factor was applied. In case all available representatives of a compartment in an organ were smaller than 40% of the grid, that particular compartment was recorded absent for that organ of the animal. BrdU-positive cells in the posterior and superficial cervical lymph nodes were also counted 'blindly' per 0.04 square mm, in the following compartments: paracortex, primary follicles, and medulla. Of every compartment, 4 of them were counted, per type of lymph node (i.e. superficial and posterior), per animal.

Statistics and principal component analysis

The statistical procedures used in the evaluation of the data were as follows: BrdU counts were analyzed by one-way analysis of covariance followed by Dunnett's multiple comparison tests;

Histopathological changes were analyzed by two-sided Fisher's exact probability test. Probability values of $p < 0.05$ were considered significant.

The rat data were analysed with multivariate analysis strategies: Principal component analysis (PCA; Jolliffe, 1986; Martens and Naes, 1989) and Principal component discriminant analysis (PCDA; Massart et al., 1997; Vandeginste et al., 1998; Hoogerbrugge et al., 1983) were performed on all BrdU parameters of NALT and lymph nodes, and on size of NALT and germinal centre development in NALT. PCA is individual-oriented and is based on the principle, that from the linear combination of the original variables other variables can be constructed. PCA describes data as a linear combination of scores and loadings. These linear combinations are called principal components (PCs). The scores and loadings vectors give a concise and simplified description of the variance present in the data. The first PC accounts for as much of the variability in the data as possible, and each succeeding PC accounts for as much of the remaining variability as possible.

PCDA, a technique which is based on the assumption that samples of the same group are more similar compared to samples of other groups, was also performed on the data in order to describe the discrimination between groups. PCDA was cross-validated by using 90% of the data to predict classification of the 10% left-out data, and by repeating the analysis until all samples were left out once.

In order to obtain a complete data set, missing values were imputed using a regularized expectation maximization (EM) algorithm. Data were autoscaled for analysis. All analyses were performed using Matlab Version 7.0.4 R14 (The Mathworks, Inc.)

Results

Atmosphere concentration

The test atmospheres were maintained constant. Analytical chamber formaldehyde concentrations measured for the 4 weeks of exposure averaged 14.96 ± 0.05 , 10.00 ± 0.39 , 6.12 ± 0.34 , 2.02 ± 0.15 , 1.00 ± 0.11 , 0.51 ± 0.05 and 0 ppm on a daily basis.

Clinical examination

During the whole study period, there were no substance-related clinical signs of toxicity in rats and mice.

The body weight development of the rats exposed to formaldehyde up to 6 ppm were comparable to the control animals. Only the body weight gain of rats exposed to 10 ppm (day 7 only) and 15 ppm were transiently decreased (-16.7 % to -32.7 %) at the beginning of the exposure period. This effect became no more statistically significant toward the end of the exposure, although the average body weight gain of the 15 ppm group was still slightly lower than in the control group.

The body weight and body weight gain of the mice, which were exposed in parallel with rats to the same concentrations of formaldehyde did not show any effect that differs from the control group.

Gross observations

Macroscopic examination at the removal of NALT from the heads of the rats and mice did not reveal remarkable gross changes. NALT had turned white, because of the fixation, and was thus clearly identifiable, but the tissue had become also somewhat brittle. In addition, NALT of mice could not be removed together with the palate as is the case with fresh tissue (Asanuma et al., 1997), but stayed *in situ*, i.e. connected to the palatine process of the maxilla

Microscopy of the H&E-stained sections of NALT and lymph nodes

Rat. Microscopic examination of the H&E-stained sections of NALT revealed FA-related slight to moderate simple hyperplasia of the lymphoepithelium of NALT in the 15 ppm group (Table 2; Figs. 3a and 3b). Although germinal centre development (a morphological parameter for activation of lymphoid tissues and organs) in NALT was not very prominent in control rats, it was completely absent in the 15 ppm group. In addition, NALT contained a decreased number of lymphoid cells (decreased cellularity) in 3 out of 8 animals of the 15 ppm group. A decreased number of lymphocytes was also observed in a single animal of the 2 ppm group. The size of NALT (morphological parameter for activation) varied considerably and ranged from very slight to marked in controls, and it did not change significantly in the exposed groups. Germinal centre development was absent in the superficial cervical lymph nodes of the 2 and 15 ppm groups.

Mouse. Microscopic examination of the H&E-stained sections of posterior and cervical lymph nodes, and NALT did not reveal FA-related histopathological changes (data not shown). The size of NALT of control mice ranged from small to large and it did not change significantly in the exposed groups. Germinal centres were scarce in NALT of both the controls and exposed animals. Germinal centres were most prominent in the superficial cervical lymph nodes, when compared to the posterior cervical lymph nodes and NALT.

Counts of BrdU-positive cells of NALT and lymph nodes

Rat. FA did not affect the number of BrdU-positive cells in the follicle and paracortex compartments and in the medulla of the superficial and posterior cervical lymph nodes (data not shown).

In NALT lymphoepithelium, the number of BrdU-positive cells was considerably increased in the 15 ppm group, when compared to the other groups including the controls (Table 3). This was a consistent finding in both series (the two series represent the two sections prepared from NALT). The number of BrdU-

positive cells varied considerably, but in both series the number of positive cells in the follicles was rather low in the 15 ppm group and to a lesser extent also in the 6 ppm group, but the differences did not reach statistical significance. This observation in combination with the lack of a concentration-response relationship suggested that FA did not influence the number of BrdU-positive cells in the follicular and interfollicular compartments.

Mouse. No FA-related effects were observed with respect to the number of BrdU-positive cells in the follicle and paracortex compartments and in the medulla of the lymph nodes; and in the follicular and interfollicular compartments and epithelium of NALT (data not shown).

Multivariate analysis (PCA and PCDA) of rat data

PCA was performed on rat data, because a few results in rats suggested an effect of FA on lower exposure groups and on parameters other than the lymphoepithelial hyperplasia at 15 ppm (i.e. the number of BrdU-positive cells were rather low in the follicles of NALT of rats in the 6 and 15 ppm groups; decreased cellularity was found in 3/8 rats of the 15 ppm group and 1/8 of the 2 ppm group). One control animal was identified as outlier, and 4 other animals (1 exposed to 0.5 ppm, one to 2 ppm and 2 exposed to 15 ppm FA) tended to be outliers. Three PC's explained 33% of the variance of the original data set. The PCA revealed that the first PC (PC1) explained a relevant amount of the variance, namely 14.2%, and the main variables/parameters responsible for the variance were those representative for lymphoepithelial proliferation (increased number of BrdU-positive epithelial cells in the epithelium above the follicles and interfollicular areas of NALT).

PCDA predicted 37.5% of the control group correctly; 12.5% of respectively the 0.5, 2 and 6 ppm groups; 0% of the 2 ppm group; 25% of the 10 ppm group and 100% of the 15 ppm group (Fig. 4). So, the

highest dose group was predicted 100% correctly, whereas no discrimination could be made between the other groups. The main variables/parameters responsible for the discrimination were those representative of lymphoepithelial proliferation.

Discussion

It has been hypothesized that, if formaldehyde inhalation exposure is related to increases in hematopoietic proliferative disorders in man, lymphomas in mice and leukemias in rats, the compound could exert such an effect via nasal leukocytes, nasal hemopoietic progenitor cells or stem cells and/or local lymphoid tissues such as nasopharynx-associated lymphoid tissue (NALT) and draining lymph nodes (Cogliano et al., 2005; Devoney, 2006; Zhang et al., 2009). The aim of the present study was to examine the effects of 28-day formaldehyde (FA) vapor exposure on NALT and the superior and posterior cervical lymph nodes, which drain the upper-respiratory tract and NALT (Wolvers et al., 1999; Wiley et al., 2005).

Seven groups of male Fischer rats and B3C3F1 mice were exposed to FA vapor at concentrations of 0, 0.5, 1, 2, 6, 10 or 15 ppm FA. Results indicated that exposure at these levels did not influence the macroscopic appearance of NALT or of the lymph nodes in rats and mice. Microscopic examination revealed treatment-related effects in NALT of rats, but not in the upper respiratory tract-draining lymph nodes of rats or in NALT and lymph nodes of mice. The absence of an effect in the upper respiratory tract-draining lymph nodes is in agreement with the results of a respiratory LLNA, in which FA was the only allergen that tested negative albeit that a relatively low concentration (3 ppm) of FA was used (Arts et al., 2008).

Microscopic examination of H&E-stained sections showed that in rats FA at 15 ppm induced simple hyperplasia of the epithelium, which covered and surrounded NALT. The increased number of BrdU-positive cells in the epithelium covering the follicles and interfollicular compartments of NALT in the 15 ppm group confirmed the hyperplasia, as observed in the H&E-stained sections. Hyperplasia of nasal epithelium is a well-known effect of FA (Woutersen et al., in press), even at levels as low as 2.0 ppm. FA induces effects from anterior to more posterior in the nasal passages with increasing concentration levels and duration of exposure (Woutersen et al., in press). Changes in NALT lymphoepithelium have been observed also with acetaldehyde (Kuper, 2006), and might be an effect that is more common with inhalation of irritating compounds than generally reported (Kuper et al, 2003).

The treatment-related hyperplasia of the lymphoepithelium of NALT at 15 ppm FA was associated with a decrease in NALT activity, i.e. absence of germinal centres in the 15 ppm group, decreased cell density in the follicles and interfollicular area (predominantly decreased number of lymphocytes, denoted as 'decreased cellularity') in a few animals, and the relatively low numbers of BrdU-positive cells in the follicles of NALT, but none of these parameters differed statistically significantly from the controls. A decreased activity of NALT might be a consequence of altered antigen-uptake and antigen-presentation by hyperplastic lymphoepithelium (Kuper, 2006). However, the decreased cellularity observed in one animal exposed to 2 ppm FA was not accompanied by epithelial hyperplasia, in which case either there may not have been a relationship between the epithelial hyperplasia and the decreased cellularity, or the decreased cellularity in the 2 and 15 ppm group may not be related to FA exposure. In addition, low numbers of BrdU-positive cells in the follicles of NALT were also found in the 6 ppm group. In order not to overlook an effect or an effect level of FA, and to examine possible correlations between parameters, PCA was performed on rat data of NALT and lymph nodes. The analysis demonstrated that the 15 ppm group of rats was the only group that differed from the others,

and in addition, that this difference depended on only one parameter, namely proliferation (BrdU-incorporation) of the lymphoepithelium of NALT.

No FA-related changes were observed in the nasal lymphoepithelium and lymphoid tissues of mice. Mice have been found to be less sensitive than rats to the epithelial damage induced by FA vapor (Swenberg et al., 1983; 1986; Kerns et al., 1983), probably because mice are able to minimize the inhalation of annoying substances more effectively than rats and have more respiratory epithelium available for filtering air per unit volume of the nasal cavity (Chang et al., 1983; Woutersen et al., 1994).

The present subacute 28-days study revealed effects of repeated exposure to a very high level (15 ppm) of FA vapor only on NALT of rats, but not of mice. If FA is related to hematopoietic malignancies in rodents, a role of NALT and upper respiratory tract-draining lymph nodes in the pathogenesis of these proliferative disorders is therefore not very plausible. Although it is rather hypothetically, it cannot be excluded that FA may act via single lymphocytes in the respiratory tract mucosa, or even via pluripotent olfactory epithelial stem cells (Zhang et al., 2009; Murrell et al., 2005). However, one would expect at least some FA-related local lymphomas, in case FA acts on local lymphoid tissues like NALT or single mucosal hematopoietic or other pluripotent stem cells.

Rather, a more systemic mechanism would be expected to increase the risk of non-local hematopoietic proliferative disorders. Such a, fairly indirect, mechanism could be through FA-related allergy or hypersensitivity. There are conflicting results with respect to associations between allergies and haematological malignancies (Merrill et al., 2007). An inverse association between malignancies and allergies was reported and explained by the Immune surveillance hypothesis: allergic conditions may lead to a decreased risk of malignancy by enhancing the ability of the immune system to detect and eliminate tumor cells. Others reported that skin hives/urticaria and asthma tended to increase the risk of leukemia, and childhood eczema tended to increase the risk of non-Hodgkin leukemia (Söderberg et

al., 2004). Such a positive association has been explained by the Antigen stimulation hypothesis: immune-stimulating conditions lead to an increased risk of haematologic malignancies. No matter if either of these associations may be valid, it is not specific for FA, but for (almost) all allergens.

It was concluded that the only distinct effect of FA vapor on NALT and local lymph nodes in Fischer 344 rats and B6C3F1 mice was simple hyperplasia of the lymphoepithelium of NALT in rats exposed to 15 ppm. Therefore, the results of the present study did not support the hypothesis that FA may induce hematological malignancies by reacting with local lymphoid tissues such as NALT.

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Legends

Fig. 1. NALT of Fischer rat *in situ*. The head was fixated in Harrison's fixative, which slightly decalcified the bones and visually enhanced lymphoid tissues like NALT.

Fig. 2. NALT of B6C3F1 mouse *in situ*. The head was fixated in Harrison's fixative, which visually enhanced NALT. The two NALT organs are located at either side of the septum (arrows).

Fig. 3. Epithelium covering NALT of a control rat (a), and the hyperplastic epithelium of a rat exposed to 15 ppm FA (b).

Fig. 4. PCDA score plot of all rats in the study. There is a difference between group 6 and the other groups. This difference is seen only in D1. D2 describes differences between individual animals instead of groups. BrdU incorporation in the epithelium above the follicles and interfollicular areas of NALT, indicative of lymphoepithelial proliferation, were the only discriminating variables/parameters. The plot did not change when outlier no. 100 was left out of the analysis.

Table 1

Study design. Exposure scheme of the rat and mouse 28-day inhalation study with FA vapor.

Test group	Concentration FA (ppm)	Concentration FA (mg/m ³)	No. of male F344 rats	No. of female B3C3F1 mice
0	-	-	8	6
1	0.5	0.6	8	6
2	1	1.25	8	6
3	2	2.49	8	6
4	6	7.5	8	6
5	10	12.5	8	6
6	15	19	8	6

Table 2

Microscopic examination of H&E-stained sections of NALT and local lymph nodes of male rats exposed to FA vapor for 4 weeks

		Incidence of lesions/changes						
		0 ppm	0.5 ppm	1 ppm	2 ppm	6 ppm	10 ppm	15 ppm
NALT								
Size								
	very small	1	0	0	1	0	2	1
	small	2	1	2	2	3	3	6
	medium	2	7	5	5	5	3	1
	large	3	0	1	0	0	0	0
Decreased cellularity								
	slight	0	0	0	0	0	0	1
	moderate	0	0	0	1	0	0	2
Germinal centre development								
	very slight	1	5	3	3	3	3	0
	slight	3	0	0	0	0	0	0
	Score expanded totals	4	5	3	3	3	3	0
Epithelial hyperplasia								
	slight	0	0	0	0	0	0	2
	moderate	0	0	0	0	0	0	5
	Score expanded totals	0	0	0	0	0	0	7**
POSTERIOR CERVICAL LYMPH NODES								
Germinal centre development								
	very slight	3	3	2	4	4	5	5
	slight	0	1	2	1	2	0	0
	moderate	1	0	1	0	0	0	0
	marked	1	0	0	0	0	0	0
	very marked	0	1	0	0	0	1	1
	Score expanded totals	5	5	5	5	6	6	6
SUPERFICIAL CERVICAL LYMPH NODES								
Germinal centre development								
	very slight	5	3	2	0	3	1	0
	very marked	0	0	1	0	0	0	0
	Score expanded totals	5	3	3	0*	3	1	0*

* $p < 0.05$; ** $p < 0.01$, Fisher's exact test between the controls and each of the treatment groups.

Table 3

BrdU counts in NALT of rats exposed to FA vapor for 4 weeks and implanted with a BrdU pump 3 days before necropsy¹

Group	ppm FA	Series 1 - Mean number of BrdU-positive cells \pm SD			
		Interfollicular area (IF)	IF epithelium	Follicular area (F)	F epithelium
0	0	61.9 \pm 18.8	6.5 \pm 3.2	73.0 \pm 39.1	12.6 \pm 17.5
1	0.5	57.3 \pm 17.4	4.9 \pm 2.2	53.5 \pm 19.4	4.9 \pm 3.8
2	1	55.7 \pm 17.7	5.9 \pm 3.4	52.2 \pm 27.9	6.4 \pm 6.5
3	2	53.5 \pm 12.9	4.3 \pm 2.7	49.8 \pm 22.1	4.7 \pm 3.2
4	6	51.1 \pm 14.9	3.3 \pm 2.4	47.6 \pm 13.9	5.8 \pm 5.3
5	10	55.5 \pm 15.3	5.5 \pm 3.5	51.2 \pm 16.2	5.7 \pm 2.9
6	15	54.4 \pm 11.6	28.2 \pm 11.1 ^{***}	41.4 \pm 14.2	23.6 \pm 13.6 [*]
		Series 2 - Mean number of BrdU-positive cells \pm SD			
0	0	48.3 \pm 17.7	6.3 \pm 2.2	62.3 \pm 24.1	6.8 \pm 1.5
1	0.5	51.0 \pm 16.3	4.4 \pm 2.7	58.0 \pm 30.5	5.8 \pm 5.6
2	1	53.9 \pm 12.2	4.1 \pm 2.9	47.0 \pm 15.3	6.9 \pm 3.8
3	2	53.4 \pm 14.2	5.1 \pm 2.4	52.2 \pm 15.1	5.6 \pm 4.0
4	6	48.2 \pm 12.3	3.5 \pm 2.3	47.2 \pm 15.0	5.9 \pm 2.8
5	10	56.0 \pm 16.3	6.4 \pm 2.3	56.8 \pm 17.4	6.2 \pm 4.7
6	15	49.9 \pm 9.1	24.5 \pm 12.6 ^{***}	40.1 \pm 11.8	22.9 \pm 10.5 ^{***}

*** p<0.001, ** p<0.01, * p<0.05, one-way ANOVA-Dunnetts' t test

¹ Counts of BrdU-positive cells were performed on 2 sets (series) of longitudinal sections of NALT. The series were counted on separate occasions.

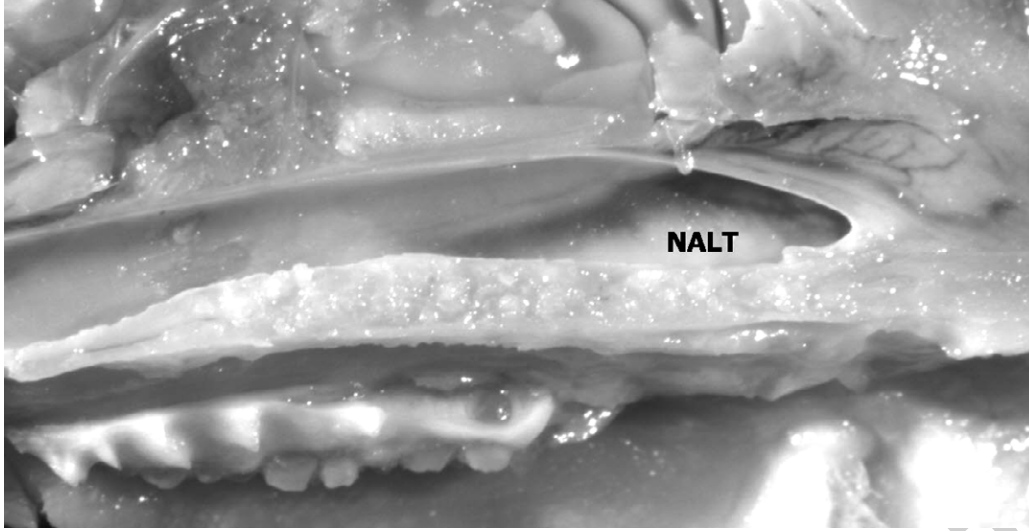


Fig. 1.

Accepted manuscript



Fig. 2

Accepted manuscript

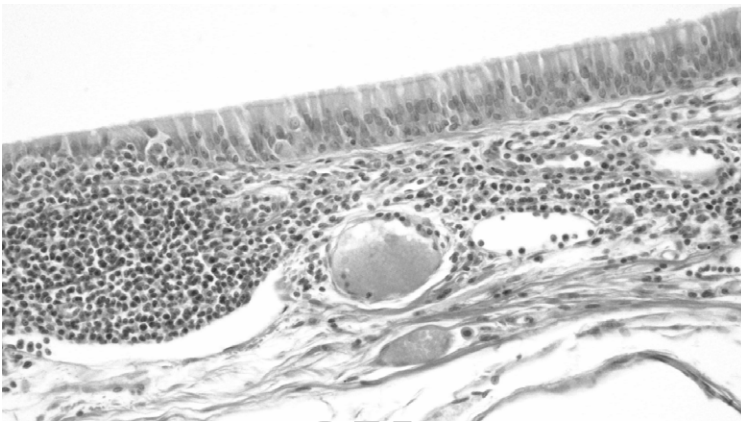
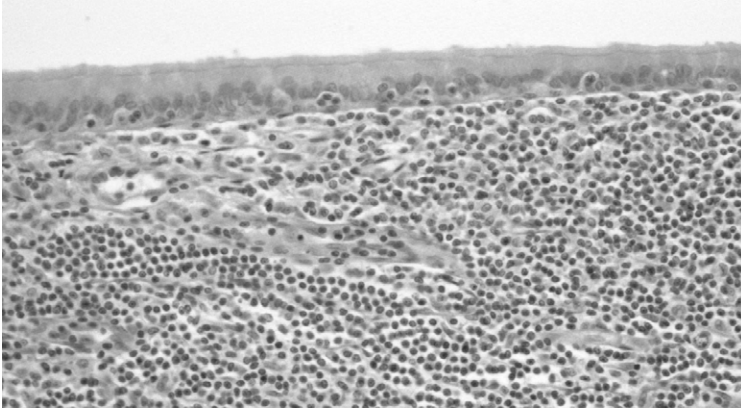


Fig. 3a (above) and **Fig. 3b** (below)

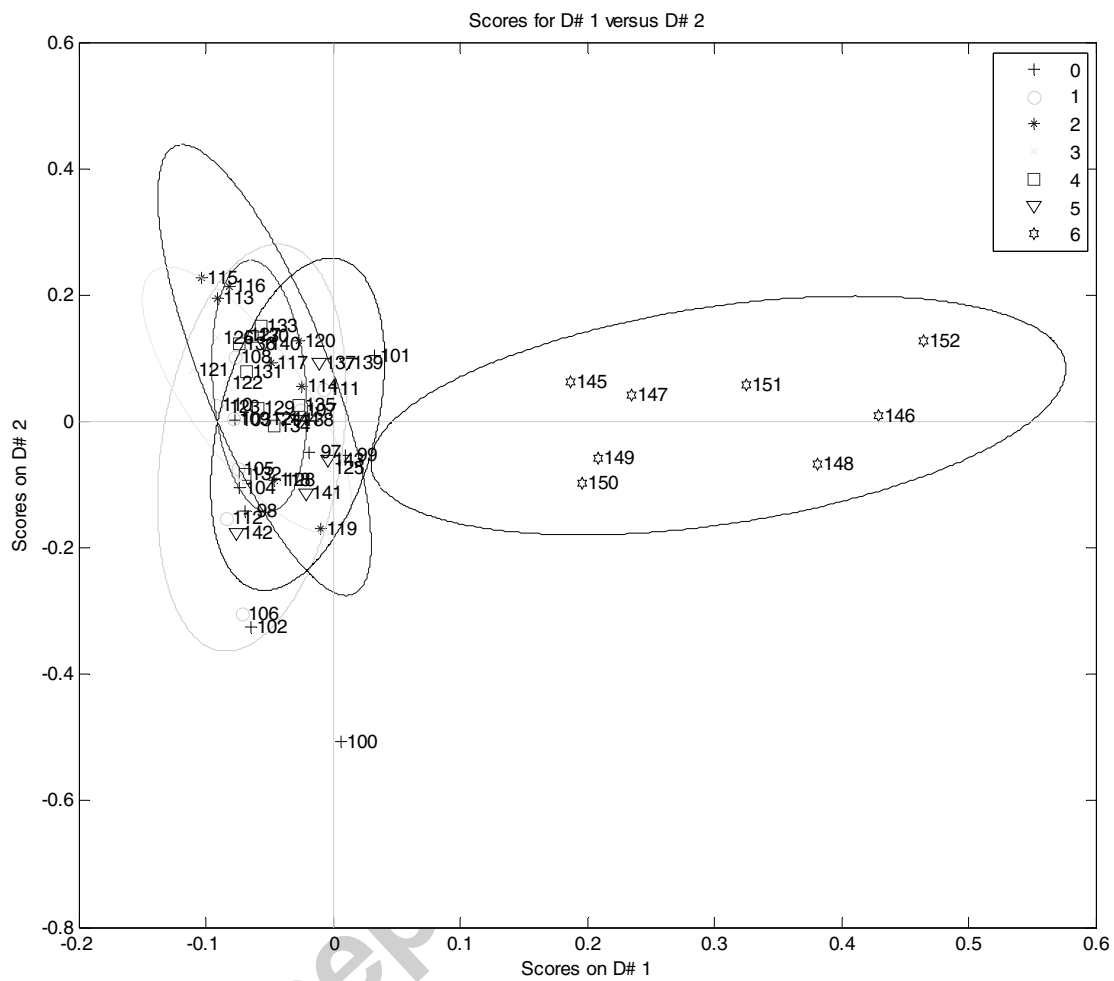


Fig. 4