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Review

Lesions in the Larynx of Wistar RccHan™: WIST Rats

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Abstract: Specific regions in the rat larynx exhibit cellular changes in response to inhaled xenobiotics. These regions include the base of the epiglottis, ventral pouch, and medial surfaces of the vocal processes of the arytenoid cartilages[1,2]. In order to collect information on the usefulness of trimming techniques, the influence of different vehicles, the impact of different application routes in toxicity studies, and differences between induced vs. spontaneous lesions, the data obtained from a large number of inhalation and non-inhalation studies performed in Wistar RCCHan™: Wist rats at Harlan Laboratories Ltd Switzerland, all evaluated or reviewed by the same pathologist, were compiled for a detailed review. The value of different trimming techniques was deemed to be greatest for transverse and sagittolongitudinal section techniques, as compared to horizontolongitudinally section techniques. The comparison of lesions encountered in control rats of inhalation studies treated with different vehicles did not reveal differences in the type, distribution pattern, incidence and/or severity of spontaneous lesions. The types of lesions were also independent of different application routes in non-inhalation studies compared to inhalation studies. The pattern of spontaneous lesions in the rodent larynx was determined by degenerative and inflammatory lesions starting most often in the submucosal glands by desiccated secretion followed by mineralization and local inflammation or were induced by impacted foreign bodies. Squamous metaplasia was recorded in the respiratory epithelium overlaying the ventral gland as a spontaneous lesion in male Wistar rats from inhalation studies with a maxim of 20.0% in an inhalation oncogenicity study. Induced metaplastic changes recorded in the larynx were reversible. Other induced lesions in inhalation studies consisted of submucosal edema, necrosis, inflammation and/or granuloma. Induced lesions in non-inhalation studies were found to be exclusively related to reflux laryngitis or food impaction. It is concluded, that in rodents induced lesions of the larynx differ in type, distribution pattern, severity and incidence from spontaneous lesions. (J Toxicol Pathol 2009; **22**: 229–246)

Key words: spontaneous laryngeal lesions, induced laryngeal lesions, Wistar RccHan™: WIST

Introduction

Although the hamster, mice, rat, rabbit, dog and monkey are species commonly used in inhalation toxicology, publication on induced and spontaneous lesion in the larynx of laboratory animals is limited. Even detailed descriptions for the histological evaluation are limited[1–5].

In order to collect information on the usefulness of trimming techniques, the influence of different vehicles, the impact of different application routes in toxicity studies, and differences between induced vs. spontaneous lesions, the authors have compiled and reviewed the data obtained from a large number of studies performed at Harlan Laboratories Ltd. Itingen during 1997–2007.

In inhalation studies, the trimming and cutting of the rat larynx according to Sagartz[5] revealed more lesions as compared to the usual longitudinal section plane applied in non-inhalation studies. In the latter technique, there is a loss of information on lesions in the ventral aspect of the larynx. In studies with certain compounds, which produce dysphagia, sagittolongitudinal sections may be advisable.

The comparison of lesions encountered in control animals of inhalation studies treated with different vehicles (air, lactose, Mg-stearate, saline), did not reveal differences in the type, distribution pattern, incidence and/or severity of spontaneous lesions. In rodents, when comparing the type of lesions in studies with different application routes, there was no difference between non-inhalation studies as compared to inhalation studies; however, the incidence completely

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depends on the different trimming techniques used for these studies.

The spontaneous lesions in the rodent larynx were found to be dominated by degenerative and inflammatory lesions starting most often in the submucosal glands in male Wistar rats from inhalation studies at earliest in 13-week studies and, at increasing incidences in studies of longer duration. These lesions were characterized by desiccated secretion followed by mineralization and local inflammation or were induced by impacted foreign bodies. Other lesions consisted of squamous metaplasia at level 6 according to Sagartz[5]. The metaplastic change recorded in the larynx of rodents is a very important issue of discussion with respect to its toxicologic significance. However, this lesion is reversible, hence, is deemed to be an indicator of the especially high sensitivity of the rodent larynx to any mild irritants.

Induced lesions in inhalation studies consisted of submucosal edema, necrosis, inflammation and/or granuloma. Induced lesions in non-inhalation studies were found to be exclusively related to reflux laryngitis or food impaction. It is concluded, that in rodents induced lesions of the larynx differ in type, distribution pattern, severity and incidence from spontaneous lesions.

Material and Methods

All the material used for this compilation was selected from studies performed in Wistar Rats at Harlan Laboratories Ltd. Itingen, Switzerland. To avoid major diagnostic shift or change in terminology, the studies selected were originally evaluated or reviewed by the same pathologist (KW) during 1997–2007. At necropsy, the larynx was sampled, fixed in 4% neutral phosphate-buffered formaldehyde (10% formalin), processed, trimmed, embedded in paraffin wax, and cut a nominal thickness of 3–4 μm. Studies in which the larynx was sectioned in a horizontolongitudinal plane are indicated in Tables 1 and 2 by 'L' followed by a study identification number, and in Table 3 the section plane was indicated by 'Longitudinal'. Studies in which the larynx was transversally sectioned according to Sagartz[5] are indicted in Tables 1 and 2 by 'T' followed by a study identification number, and in Table 3 the section plane was indicated by the 'Level Number'. The different possibilities of section planes are demonstrated in Fig. 1. Relevant information for each of the studies used for this compilation is summarized in Tables 1 and 2, including an indication of studies where induced laryngeal lesions were recorded. For the evaluation of the incidence of spontaneous lesions, only control animals were used for calculations. In all other studies, there were no differences recorded between control and test item-treated animals, hence, all study animals were taken into consideration.

Results

Spontaneous or induced lesions of the larynx were generally infrequently recorded in rats. Moreover, these lesions appeared to follow a very stereotypic pattern. Lesions were recorded in studies regardless of study duration. Spontaneous and induced lesions did not differ significantly in type, distribution, and nature when comparing short term and chronic studies.

Trimming technique (Fig. 1)

For the detection of lesions, the cutting planes were

Table 1. General Information on Evaluated Inhalation Studies Performed in Wistar RccHan™: WIST Rats Including Duration, Vehicle, Number of Main Test and Recovery Groups, Number of Main Test and Recovery Animals, and Indication of Studies with Induced Lesions

Acute: Inhalation: Study ID	Vehicle	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
L1	Lactose	4	–	5	–
L2**	Air	5	–	10	–
L3**	Air	2	–	5	–
L4**	Lactose	4	–	5	–
L5**	Air	2	–	6	–
L6**	Lactose	5	–	5	–
L7**	Lactose	5	–	5	–
L8**	Air	4	–	8	–
L9**	Lactose	4	–	8	–
T1	Air	4	–	5	–
T2	Lactose	4	–	5	–
T3*, ***	Saline	4	–	10	–
T4*	Mg-Stearate/Lactose	4	–	5	–
T5***	Lactose	3	–	5	–

* T3: oedema, inflammation, glandular dilation, squamous metaplasia, keratinization at low to high dose; T4: squamous metaplasia, inflammation at low to high dose. ** No lesion noted in study. *** Males only investigated.

Table 1. continued

14-Day: Inhalation Study ID	Vehicle	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
L1	Lactose	5	–	10	–
L2**	Lactose	4	–	10	–
L3	Air	4	–	10	–
L4*	Lactose	4	–	5	–
L5**	Lactose	4	–	5	–
L6**	Lactose	5	–	5	–
T1	Placebo Aerosol	5	–	10	–
T2*	Lactose	3	3	10	10
T3	Lactose	4	2	10	5

* L4: Squamous metaplasia, inflammation at low to high dose; T2: squamous metaplasia at low to high dose. ** No lesion noted in study. L: Longitudinal section, T: Transversal section.

4-Week: Inhalation Study ID	Vehicle	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
L1	Lactose, Mg-Stearate	6	3	10	5
L2**	Lactose	4	–	12	–
L3**	Air, Lactose	6	–	10	–
L4**	Saline	4	–	10	–
L5wb**	Air	4	–	10	–
L6**	Air	4	–	10	–
L7	Lactose	4	–	10	–
L8**	Lactose	5	–	10	–
L9*	Lactose	4	–	5	–
T1	Air	5	2	12	8
T2*	Mg-Stearate/Lactose	5	5	12	8
T3	Air	4	4	12	8
T4	Air, Lactose	5	5	10	5
T5	Air	4	–	5	–
T6*	Air	4	–	10	–
T7*	Saline	6	–	10	–
T8	Lactose	5	2	10	5

* T2: squamous metaplasia in groups 4–5, T6: erosion, squamous metaplasia at high dose, T7: necrosis, inflammation, squamous metaplasia in groups at low to high dose, L9: increased incidence of glandular dilation and mononuclear foci, degeneration/necrosis of respiratory epithelium, squamous metaplasia at low to high dose. ** No lesion noted in study. L: Longitudinal section, T: Transversal section.

13-Week: Inhalation Study ID	Vehicle	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
L1	Saline	5	3	10	5
L2	?	5	3	10	5
T1*	Air, Lactose	5	5	12	6
T2	Air	4	–	10	–
T3	Placebo Aerosol	4	4	10	5
T4	Air, Placebo	5	3	10	5
T5*	Air, Placebo	5	5	10	5
T6*	Air	4	2	10	10
T7*	Lactose	2	0	10	10

* T1: squamous metaplasia at high dose, T5: squamous metaplasia at low to high dose, T6: squamous metaplasia at high dose, T7: squamous metaplasia at high dose. L: Longitudinal section, T: Transversal section.

Table 1. continued

26-Week: Inhalation Study ID	Vehicle	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
T1*	Lactose	2	2	40	20
T2*	Air, Lactose	5	5	20	10
T3*	Air, Lactose	5	5	20	10
T4	Lactose	5	5	10	5
T5*	Lactose	4	4	20	10
T6	Lactose	4	4	20	10
104-Week: Inhalation					
Study ID	Vehicle	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
T1**	Lactose	2	–	62	–

* T1; squamous metaplasia, epitehlial disorganization at high dose, T2: squmous metaplasia at high dose, T3: squamous metaplasia at low to high dose, T5: squamous metaplasia, keratosis, fibrosis at low to high dose. ** T1; squamous metaplasia. L: Longitudinal section, T: Transversal section.

Table 2. General Information on Evaluated Non-Inhalation Studies Performed in Wistar RccHan™: WIST Rats Including Duration, Route of Application, Number of Main Test and Recovery Groups, Number of Main Test and Recovery Animals, and Indication of Studies with Induced Lesions

14-Day: Non-Inhalation Study ID	Route of application	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
1	Gavage	4	–	–/10	–
2	Gavage	2	–	8	–
3	Gavage	2	–	10	–
4	Gavage	2	–	10	–
5*	Gavage	2	–	10	–
6	Intra-ocular	2	2	10	5
7	Gavage	2	–	6	–
8	Intra-venous	2	–	10	–
9	Gavage	2	–	5	–
10	Gavage	4	2	10	5
11	Intra-venous	2	–	10	–

* Regurgitation laryngitis.

considered to be decisive. In horizontolongitudinal sections planes, lesions were rarely recorded resulting in low incidences. In non-inhalation studies, this technique revealed non-neoplastic lesions only in cases of induced regurgitation laryngitis. A few lesions were present in oncogenicity studies and almost no lesions were observed in all remaining studies evaluated. Even in inhalation studies, this trimming technique revealed a low number of positive findings. In 88.9% of all acute inhalation studies, in 50% of all 14-day inhalation studies, and in 75.0% of all 4-week inhalation studies that were evaluated based on horizontolongitudinal sections, no lesions were recorded, versus in 100% of all studies of these durations evaluated by transversal sections lesions.

In 13-week studies, the only finding recorded in horizontolongitudinal sections consisted of mononuclear cell foci in the submucosa. There were only one 14-day study and one 4-week study, evaluated in horizontolongitudinal sections that were positive regarding induced lesions whereas in studies evaluated by transversal sections, 40% of acute studies, 33.3% of 14-day studies, 37.5% of 4-week studies, and 57.1% of 13-week studies had positive findings. Furthermore, in 66.7% of all 26-week studies evaluated by transversal sections induced lesions were recorded.

Spontaneous lesions recorded in inhalation studies

In studies evaluated based on transversal sections, the

Table 2. continued

4-Week: Non-Inhalation Study ID	Route of application	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
1	Intra-venous	2	2	10	5
2	Gavage	2	2	10	10
3	Gavage	2	–	10	–
4	Dermal	3	–	10	–
5	Gavage	2	2	10	5
6	Gavage	2	2	5	5
7	Gavage	2	2	10	5
8	Gavage	2	2	5	5
9	Gavage	2	–	5	–
10	Dermal	7	–	10	10
11	Gavage	2	–	5	–
12	Gavage	2	–	5	–
13	Gavage	2	–	5	–
14	Gavage	2	2	5	5
15	Dermal	2	–	5	–
16	Gavage	2	–	5	–
17	Gavage	2	–	10 (24)**	–
18*	Gavage	4	2	10	5
19	Gavage	2	–	5	–
20	Gavage	2	–	5	–
21	Gavage	4	2	10	5
22	Gavage	6	4	10	5
23	Gavage	4	–	5	–
24	Gavage	2	2	10	4

* Regurgitation laryngitis. ** 24 animals per sex in high dose group.

13-Week: Non-Inhalation Study ID	Route of application	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
1	Gavage	2	2	15	5
2	Gavage	2	2	10	5
3	Feeding	2	2	10	5
4	Gavage	2	2	10	5
5	Feeding	2	–	10	–
6	Gavage	2	2	30	15
7	Feeding	2	–	12	–
8	Gavage	2	2	10	5
9	Gavage	2	–	10	–
10	Feeding	2	2	10	5
11	Gavage	2	2	10	5
12	Feeding	2	2	10	5
13	Feeding	2	–	12	–
14	Gavage	2	2	10	5
15	Gavage	2	2	10	5

spectrum of lesions recorded ranged from intraluminal hemorrhage as an agonal artifact to the deposition of foreign bodies consisting mainly of food remnants and in rare cases of hairs (Fig. 2). This may lead to the formation of granuloma or chronic active inflammation of the ventral diverticulum (Fig. 3). Desiccation of glandular secretion appearing as a grayish to basophilic mass within dilated ducts of the submucosal sero-mucinous glands mainly in levels 2 to 4 was one of the most frequently recorded lesions. This desiccation was often present along with partial or complete mineralization in the same or adjacent glandular ducts in levels 2–4 (Fig. 4). The range of inflammatory lesions consisted of mononuclear cell foci that were recorded in the submucosa of any laryngeal level without any predilection sites or preferred age, and rarer acute (Fig. 5) to chronic or chronic active (Fig. 6) glandular inflammation. These types of inflammation were most often recorded in levels 2 and 3. Acute inflammatory processes consisted of

Table 2. continued

26-Week: Non-Inhalation Study ID	Route of application	Number of main test groups examined	Number of recovery groups examined	Main test animals per sex	Recovery animals per sex
1	Gavage	2	2	20	10
2	Dermal	2	2	10	15
3	Subcutaneous	2	2	10	10
4*	Gavage	2	2	20	10
52-Week: Non-Inhalation					
1	Feeding	2	2	20	5
2	Feeding	2	–	20	–
104-Week: Non-Inhalation					
1	Feeding	2	–	70**	–
2	Feeding	5	–	50	–
3	Feeding	5	–	70**	–
4	Feeding	5	–	50	–

* Induced impaction of food material in high dose. ** Interim sacrifice of 20 animals per sex after 52 weeks.

Table 3. Relative Incidences of Spontaneous Lesions Recorded in the Larynx of Wistar RccHan™: WIST Rats from Inhalation Studies (Mean, Standard Deviation, Minimum, Maximum)

Section plane/Lesion per study type	Male (n=26)				Female (n=26)			
Acute inhalation studies	Mean	SD	Min	Max	Mean	SD	Min	Max
Longitudinal: Mononuclear cell foci	6.7	18.9	0	60.0	3.3	9.4	0	30.0
Longitudinal: Inflammation	1.1	3.1	0	10.0	0	0	0	0
Level 2: Foreign bodies	1.3	2.7	0	6.7	0	0	0	0
Level 2: Inflammation	4.0	8.0	0	20.0	0	0	0	0
Level 3: Foreign bodies	1.3	2.7	0	6.7	0	0	0	0
Level 3: Desiccation	4.0	8.0	0	20.0	0	0	0	0
Level 3: Mononuclear foci	1.0	2.0	0	5.0	1.7	2.4	0	5.0
Level 4: Foreign bodies	3.4	3.4	0	6.7	0	0	0	0
Level 4: Mononuclear cell foci	0	0	0	0	20.0	0	20.0	20.0
Level 5: Mononuclear cell foci	20.0	20.0	0	40.0	20.0	0	20.0	20.0
Level 6: Squamoid epithelium	21.0	26.2	0	60.0	40.0	43.2	0	100
Level 6: Glandular dilation	20.0	40.0	0	100	0	0	0	0
Level 6: Mononuclear cell foci	8.0	16.0	0	40.0	3.3	4.7	0	10.0

edema with scattered inflammatory cells, mainly granulocytes, plasma cells and rare mast cells (Fig. 7). Along with inflammation in 26-week and oncogenicity studies, there were single cases of respiratory epithelial hyperplasia (Fig. 8). Another rare lesion was hemosiderin deposition in the submucosa in levels 2 and 3 in the 26-week and oncogenicity studies.

Amongst spontaneous lesions, squamous metaplasia with epithelial keratinization, mainly in the respiratory epithelium overlaying the ventral gland at level 6, was present in one case in a 13-week study. The metaplasia consisted of replacement of the original cuboidal epithelial cells by a diffused stratified (2–3 and more cell layers) squamous epithelium whereby the mucosal height was increased as compared to the normal respiratory epithelium. At level 6, the normal respiratory epithelium is often squamoid in appearance (Fig.9). In order to avoid misinterpretation and confusion with squamous metaplasia, the incidence of the normal squamoid epithelium was recorded in Table 3. It is a common respiratory epithelial appearance at this site and ranges (mean) from 10.3% to 40% depending on sex and study duration. Squamous metaplasia was also recorded at the ventrolateral aspects of levels 2, 3 and 5 in 4-week to 26-week studies.

Table 3. continued

14-Day Inhalation studies	Male (n=43)				Female (n=43)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Longitudinal: Glandular dilation	16.7	37.3	0	100	16.3	36.5	0	98.0
Longitudinal: Mononuclear cell foci	7.4	15.5	0	42.0	5.9	10.2	0	28.0
Longitudinal: Granuloma	1.2	2.8	0	7.5	0.4	0.9	0	2.5
Level 2: Haemorrhage	1.1	1.6	0	3.3	0	0	0	0
Level 2: Desiccation	3.3	4.7	0	10.0	1.7	2.4	0	5.0
Level 2: Glandular inflammation	3.3	4.7	0	10.0	0	0	0	0
Level 3: Haemorrhage	1.1	1.6	0	3.3	0	0	0	0
Level 3: Desiccation	10.0	14.1	0	30.0	6.7	9.4	0	20.0
Level 3: Glandular inflammation	3.3	4.7	0	10.0	1.7	2.4	0	5.0
Level 4: Desiccation	20.0	0.0	20.0	20.0	30.0	0	30.0	30.0
Level 4: Mononuclear cell foci	10.0	0	10.0	10.0	15.0	0	15.0	15.0
Level 4: Glandular inflammation	0	0	0	0	5.0	0	5.0	5.0
Level 5: Foreign Bodies	5.0	0	5.0	5.0	0	0	0	0
Level 5: Mononuclear cell foci	10.0	0	10.0	10.0	20.0	0	20.0	20.0
Level 5: Granuloma	5.0	0	5.0	5.0	0	0	0	0
Level 6: Squamoid epithelium	20.6	18.6	0	45.0	37.2	26.3	0	56.7
Level 6: Haemorrhage	1.1	1.6	0	3.3	0	0	0	0
Level 6: Squamous metaplasia	1.7	2.4	0	5.0	3.3	4.7	0	10.0

4-Week inhalation studies	Male (n=159)				Female (n=159)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Longitudinal : Squamous metaplasia	0.2	0.5	0	1.4	0	0	0	0
Level 2: Foreign bodies	0.5	0.9	0	2.5	0	0	0	0
Level 2: Haemorrhage	0.2	0.4	0	1.3	0	0	0	0
Level 2: Glandular dilation	8.8	23.2	0	70.0	6.9	18.2	0	55.0
Level 2: Desiccation	0.9	1.7	0	5.0	0.3	0.8	0	2.5
Level 2: Mineralization	1.6	3.3	0	10.0	0.6	1.7	0	5.0
Level 2: Mononuclear cell foci	3.1	8.3	0	25.0	2.4	4.9	0	15.0
Level 2: Glandular inflammation	0.3	0.8	0	2.5	0	0	0	0
Level 2: Ventral pouch inflammation	0.3	0.9	0	2.7	0.2	0.4	0	1.3
Level 3: Foreign bodies	0.8	1.6	0	5.0	0	0.0	0	0
Level 3: Glandular dilation	1.2	3.3	0	10.0	1.2	3.3	0	10.0
Level 3: Desiccation	1.6	3.3	0	10.0	1.2	2.5	0	7.5
Level 3: Mononuclear cell foci	10.1	21.0	0	65.0	12.6	27.6	0	85
Level 3: Glandular inflammation	0.9	1.7	0	5.0	0.2	0.4	0	1.2
Level 3: Granuloma	0.6	1.1	0	2.5	0	0.0	0	0
Level 3: Squamous metaplasia	2.5	6.6	0	20.0	3.1	8.3	0	25.0
Level 4: Foreign bodies	0.8	1.2	0	2.5	0	0.0	0	0
Level 4: Haemorrhage	0.8	1.2	0	2.5	0	0.0	0	0
Level 4: Desiccation	0.8	1.2	0	2.5	0.8	1.2	0	2.5
Level 4: Mononuclear cell foci	10.0	10.8	0	25	2.5	2.0	0	5.0
Level 4: Granuloma	2.5	2.0	0	5.0	0	0.0	0	0
Level 5: Squamoid epithelium	0	0	0	0	0.8	1.2	0	2.5
Level 5: Haemorrhage	0.8	1.2	0	2.5	0	0.0	0	0
Level 5: Mononuclear foci	10.0	9.4	0	22.5	10.0	10.8	0	25.0
Level 5: Granuloma	0.8	1.2	0	2.5	0	0.0	0	0
Level 6: Squamoid epithelium	15.9	21.1	0	50.0	10.3	18.0	0	45.0
Level 6: Haemorrhage	0.31	0.8	0	2.5	0	0.0	0	0
Level 6: Mineralization	0.2	0.4	0	1.3	0	0	0	0
Level 6: Mononuclear cell foci	4.38	6.8	0	20.0	5.6	13.1	0	40.0
Level 6: Squamous metaplasia	0	0	0	0	0.3	0.8	0	2.5

Table 3. continued

13-Week inhalation studies	Male (n=150)				Female (n=150)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Longitudinal: Mononuclear foci	4.6	4.6	0	9.2	6.2	0.0	6.2	6.2
Level 2: Haemorrhage	0.7	1.7	0	5.0	0	0	0	0
Level 2: Desiccation	1.2	1.9	0	5.0	4.3	6.8	0	20.0
Level 2: Mononuclear foci	7.7	13.2	0	38.9	2.2	3.7	0	10.0
Level 2: Glandular inflammation	4.7	9.6	0	27.8	2.3	4.0	0	11.1
Level 2: Squamous metaplasia	1.4	3.5	0	10.0	0	0	0	
Level 3: Foreign bodies	0.6	1.5	0	4.4	0.3	0.8	0	2.2
Level 3: Haemorrhage	0.7	1.7	0	5.0	0	0	0	0
Level 3: Desiccation	2.4	4.6	0	13.3	7.1	10.9	0	30.0
Level 3: Mineralization	0.2	0.6	0	1.7	2.4	5.2	0	15.0
Level 3: Mononuclear cell foci	12.9	18.0	0	52.8	7.3	7.3	0	22.2
Level 3: Glandular inflammation	0.7	1.1	0	2.8	1.0	1.8	0	5.0
Level 3: Ventral pouch inflammation	0.7	1.7	5.0	0	0	0	0	
Level 3: Granuloma	0.4	1.0	0	2.8	0	0	0	0
Level 3: Squamous metaplasia	1.4	3.5	0	10.0	0	0	0	0
Level 4: Foreign bodies	2.0	1.5	0	3.3	0	0	0	0
Level 4: Desiccation	11.7	8.5	0	20	5.6	4.2	0	10.0
Level 4: Mineralization	1.7	2.4	0	5.0	5.0	7.1	0	15.0
Level 4: Mononuclear cell foci	36.3	26.3	10.0	72.2	34.8	24.6	15.0	69.4
Level 4: Granuloma	0.9	1.3	0	2.8	0	0	0	0
Level 5: Squamoid epithelium	1.6	1.7	0	3.3	0	0	0	0
Level 5: Foreign bodies	0	0	0	0	1.4	1.4	0	2.8
Level 5: Glandular dilation	1.6	1.7	0	3.3	0	0	0	0
Level 5: Mononuclear cell foci	58.9	2.2	56.7	61.1	31.7	1.7	30.0	33.3
Level 6: Squamoid epithelium	13.3	18.5	0	53.3	13.6	16.6	0	40.0
Level 6: Foreign bodies	0	0	0	0	0.4	1.0	0	2.8
Level 6: Haemorrhage	0.7	1.7	0	5.0	0	0	0	0
Level 6: Mineralization	0.7	1.7	0	5.0	0	0	0	0
Level 6: Mononuclear cell foci	10.2	15.4	0	45.0	3.2	5.0	0	11.1
Level 6: Squamous metaplasia	0.5	1.2	0	3.3	2.3	3.4	0	10.0
Level 6: Epithelial keratinization	0.3	0.8	0	2.2	0	0	0	0

Based on transversal sections, there were higher incidences of impacted food material/foreign bodies in males as compared to females in acute to 13-week studies, but not thereafter. Similarly, desiccation of glandular secretion (mainly in the posterior larynx levels 2 and 3, but not in level 4 from 14-day studies) was noted at higher incidences in males from acute to 13-week studies along with glandular inflammation. In single cases, ventral pouch inflammation or granuloma formation (14-day to 4-week studies) was also evident. Mineralization accompanying desiccated glandular secretion was more often recorded in females in levels 2, 3 and/or 4 in 4-week and 13-week studies.

These differences were less evident in 13-week and longer studies. Spontaneous squamous metaplasia of the mucosa overlaying the ventral aspect of the larynx at level 6 first became evident in 14-day studies with a higher incidence in females. This higher incidence in females was recorded in studies up to 13 weeks, and thereafter, squamous metaplasia at level 6 was recorded at higher incidences in males. All remaining spontaneous lesions recorded did not reveal significant differences between males and females.

In studies evaluated by horizontolongitudinal sections spontaneous lesions were rare and consisted of dilated submucosal sero-mucinous glands, submucosal mononuclear cell foci, as well as, single cases of submucosal/mucosal inflammation, granuloma formation, or even squamous metaplasia at the aperture (aditus laryngis) in one case. No significant differences were noted in sex or time course of the studies.

Induced lesions recorded in inhalation studies

The most common induced lesion consisted of squamous metaplasia of the ventral respiratory epithelium at level 6 overlaying the ventral gland. It was recorded in all 16 positive studies, whereas in 9 studies (56.2%) it was the only

Table 3. continued

26-Week inhalation studies	Male (n=62)				Female (n=62)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Level 2: Glandular dilation	8.8	19.6	0	52.5	8.3	18.6	0	50.0
Level 2: Desiccation	6.4	6.6	0	16.7	8.1	9.4	0	26.7
Level 2: Glandular inflammation	0	0	0	0	0.6	1.2	0	3.3
Level 2: Mineralization	2.2	3.7	0	10.0	4.4	6.6	0	16.7
Level 2: Hemosiderin	0.8	1.9	0	5.0	0.55	1.2	0	3.3
Level 2: Mononuclear cell foci	4.2	7.3	0	20.0	1.4	2.0	0	5.0
Level 2: Inflammation, sub-/mucosa	0	0	0	0	1.7	3.7	0	10.0
Level 3: Foreign bodies	0.6	1.2	0	3.3	1.1	2.5	0	6.7
Level 3: Glandular dilation	0.1	0.3	0	0.8	0	0	0	0
Level 3: Desiccation	18.9	21.3	0	50.0	17.5	20.6	0	56.7
Level 3: Mineralization	5.6	9.6	0	26.7	5.6	9.8	0	26.7
Level 3: Mononuclear cell foci	15.6	13.8	1.7	43.3	12.4	10.6	0	33.3
Level 3: Glandular inflammation	6.4	9.6	0	26.7	0.6	1.2	0	3.3
Level 3: Ventral pouch inflammation	0	0	0	0	1.7	3.7	0	10.0
Level 3: Granuloma	0	0	0	0	1.1	1.6	0	3.3
Level 3: Squamous metaplasia	0.8	1.9	0	5.0	0.8	1.9	0	5.0
Level 3: Respiratory hyperplasia	0.3	0.6	0	1.7	0	0	0	0
Level 4: Desiccation	13.3	0	13.3	13.3	6.7	0	6.7	6.7
Level 4: Mononuclear foci	46.7	0	46.7	46.7	70.0	0	70.0	70.0
Level 5: Glandular dilation	1.6	1.7	0	3.3	1.6	1.7	0	3.3
Level 5: Mononuclear cell foci	36.7	30.0	6.7	66.7	29.2	24.2	5.0	53.3
Level 5: Squamous metaplasia	2.5	2.5	0	5.0	0	0	0	0
Level 6: Squamoid epithelium	35.8	34.0	0	93.3	33.9	35.6	0	100
Level 6: Foreign bodies	0.3	0.6	0	1.7	0.3	0.6	0	1.7
Level 6: Haemorrhage	0	0	0	0	1.1	2.5	0	6.7
Level 6: Mineralization	0.1	0.3	0	0.8	0	0	0	0
Level 6: Mononuclear cell foci	9.9	13.8	0	36.7	10.1	13.6	0	34.2
Level 6: Glandular inflammation	1.1	2.5	0	6.7	1.1	2.5	0	6.7
Level 6: Inflammation, sub-/mucosa	0.8	1.3	0	3.3	0.7	1.2	0	3.3
Level 6: Granuloma	0.6	1.2	0	3.3	0	0	0	0
Level 6: Squamous metaplasia	2.2	5.0	0	13.3	2.2	3.7	0	10.0

104-Week inhalation studies	Male (n=62)				Female (n=62)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Level 2: Foreign bodies	0	0	0	0	0.8	0	0.8	0.8
Level 2: Hemorrhage	0.6	0	0.6	0.6	0.8	0	0.8	0.8
Level 2: Mineralization	4.2	0	4.2	4.2	11.7	0	11.7	11.7
Level 2: Inflammation, sub-/mucosa	6.7	0	6.7	6.7	0.8	0	0.8	0.8
Level 3: Haemorrhage	0.8	0	0.8	0.8	0.8	0	0.8	0.8
Level 3: Dessication	3.3	0	3.3	3.3	0	0	0	0
Level 3: Mineralization	0	0	0	0	7.5	0	7.5	7.5
Level 3. Hemosiderin	0	0	0	0	0.8	0	0.8	0.8
Level 3: Mononuclear cell foci	1.7	0	1.7	1.7	1.7	0	1.7	1.7
Level 3: Inflammation, sub-/mucosa	0.8	0	0.8	0.8	1.7	0	1.7	1.7
Level 3: Granuloma	0.8	0	0.8	0.8	0	0	0	0
Level 3: Respiratory hyperplasia	1.7	0	1.7	1.7	0	0	0	0
Level 6: Haemorrhage	15.0	0.0	15	15	5	0.0	5	5
Level 6: Squamous metaplasia	20.1	0.0	20.1	20.1	7.5	0.0	7.5	7.5
Level 6: Granuloma	1.7	0.0	1.7	1.7	0	0.0	0	0

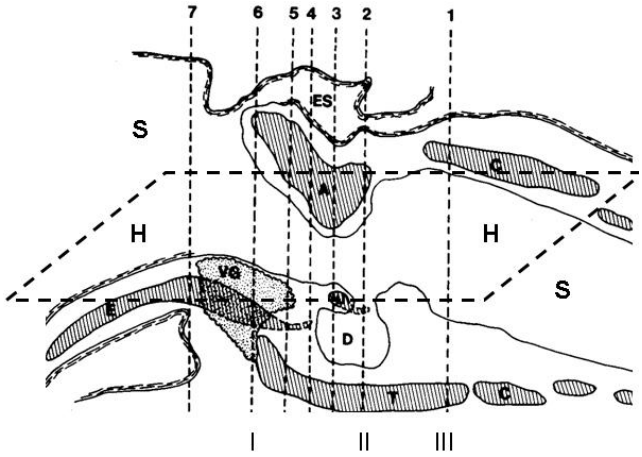

Fig. 1. Diagram on possible and published sections plans of the rat larynx. 1–7: transversal sections according to Sagartz[5]. I–III: transversal sections according to RITA[8]. H: horizontolongitudinal section. S: sagittolongitudinal section.

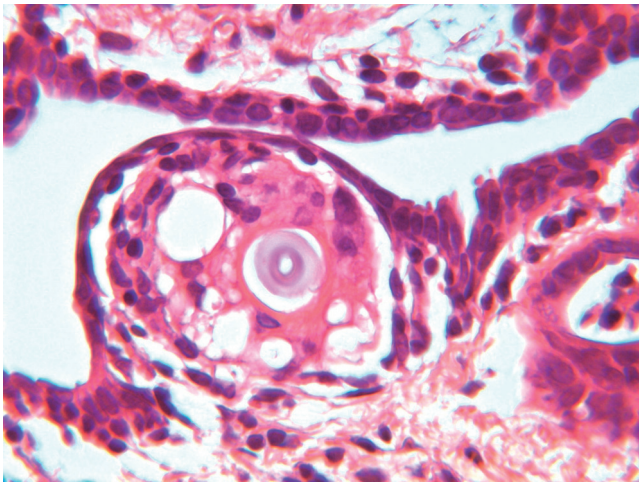

Fig. 2. Foreign body granuloma with multinuclear giant cells protruding into laryngeal lumen, probably induced by hair. Level 2[5]. HE, lens × 100.

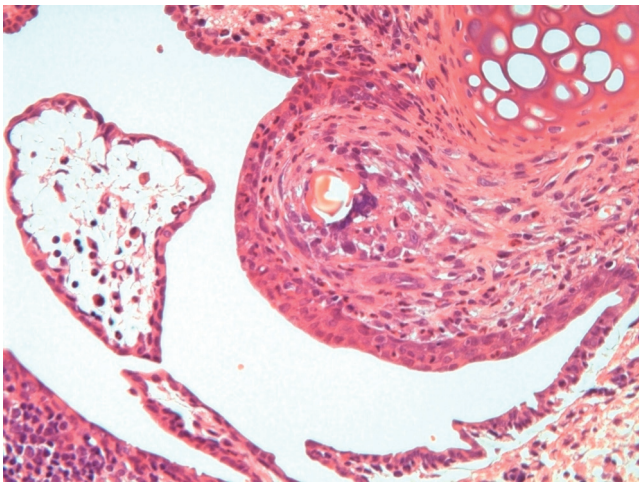

Fig. 3. Foreign body granuloma and related chronic active inflammation of the ventral diverticulum. Level 4[5]. HE, lens × 100.

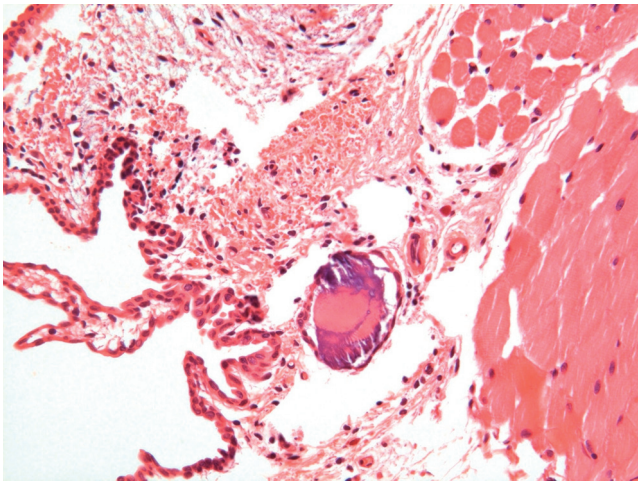

Fig. 4. Desiccated secretion in dilated duct of submucosal sero-mucinous glands partially mineralized. Level 2[5]. HE, lens × 40.

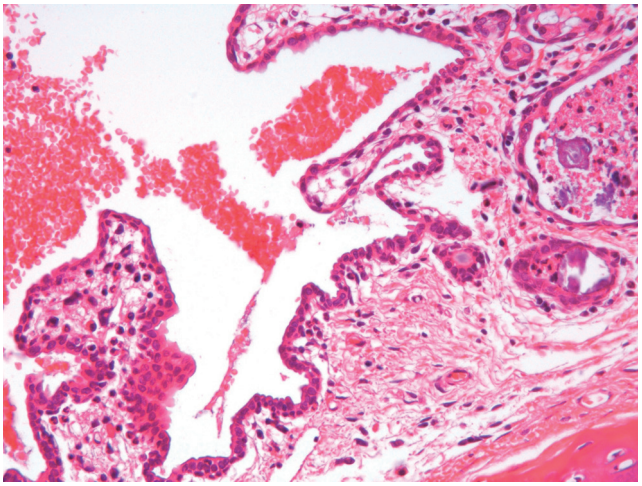

Fig. 5. Acute inflammation of submucosa and submucosal glandular ducts. Note mineralizations in dilated duct of sero-mucinous glands. Level 2[5]. HE, lens × 40.

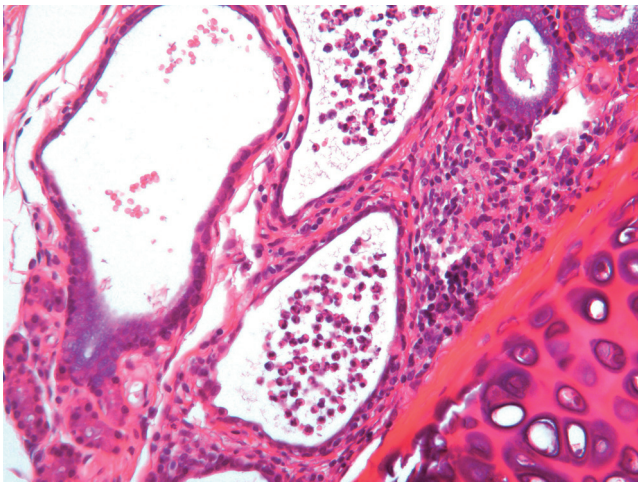

Fig. 6. Chronic active inflammation of submucosa and submucosal sero-mucinous glands. Level 2[5]. HE, lens × 40.

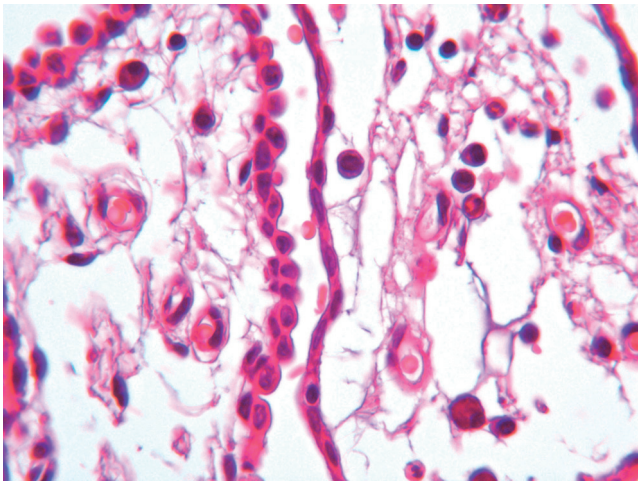

Fig. 7. Subacute inflammation of submucosa. Scattered inflammatory cells characterized by granulocytes, plasma cells and lymphocytes. Level 2^5. HE, lens × 100.

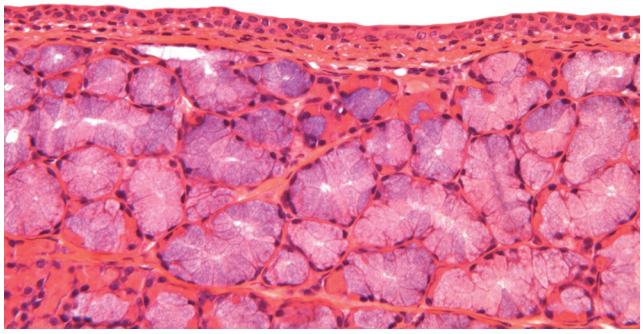

Fig. 9. Squamoid epithelium is a normal variant of respiratory epithelium overlaying the ventral gland. Level 6^5. HE, lens × 40.

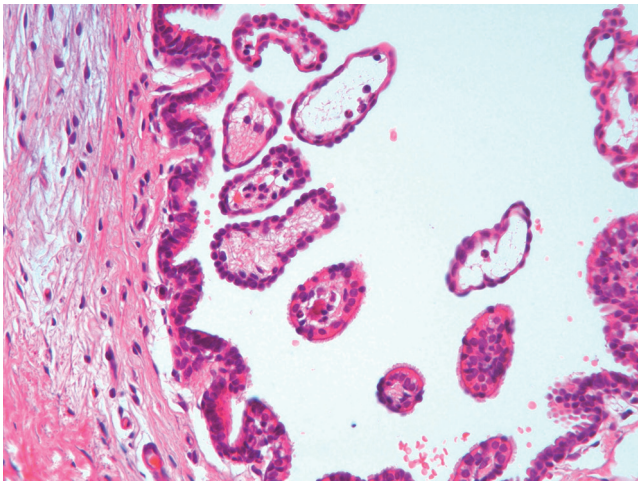

Fig. 8. Subacute inflammation of submucosal and mucosa along with hyperplasia of respiratory epithelium. Level 2^5. HE, lens × 40.

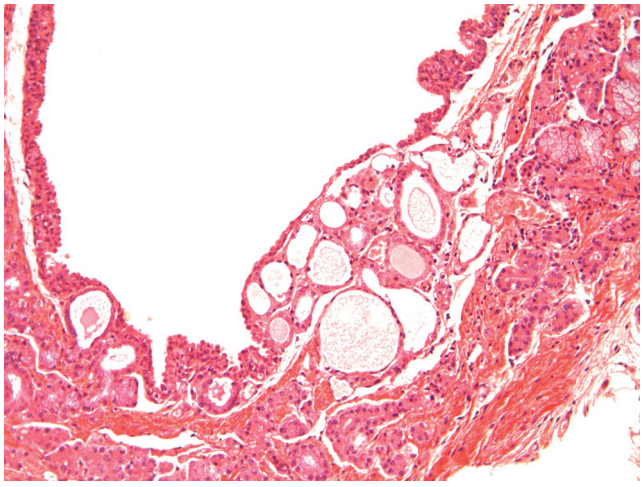

Fig. 10. Induced focal dilation of submucosal sero-mucinous glands. Level 2^5. HE, lens × 20.

lesion recorded. This lesion does not differ from the spontaneous occurring squamous metaplasia except in incidence. In a single study (26-week study T1), there were a few cases of epithelial disorganization adjacent to the metaplastic squamous epithelial layer. When only squamous metaplasia was recorded as an induced lesion, there was almost complete recovery after a 2-week treatment-free recovery period in 4-week studies (except study T2) and after a 4-week treatment-free period in 13-week studies (except study T6). In the 26-week study that showed epithelial disorganization adjacent to the metaplastic squamous epithelial layer (26-week study T1) complete recovery was also present. In oncogenicity studies, the only induced lesion was squamous metaplasia at level 6 without any indicator for dysplasia or hyperplasia.

Inflammatory lesions recorded in an acute study (acute study T3) ranged from focal or multifocal dilation of submucosal glands mainly at levels 2 and 3 (Fig. 10), submucosal edema with scattered inflammatory cells, mainly granulocytes and plasma cells (Fig. 11) and/or submucosal subacute inflammation in levels 2, 3 and 6 (Fig. 12). In the acute to subacute phase of inflammation the loss of cilia on the respiratory epithelium took place (Fg. 13). In this study, squamous metaplasia with hyperplasia and keratinization was recorded at the arytenoids projections at level 3 (Fig. 14). In two studies, focal erosions of the epithelium (4-week study T6) or necrosis (4-week study T7) of large portions of the lining epithelium (Fig. 15, 16) without preference of an epithelial type were present. In the first study, the erosions were observed in single animals of

Fig. 11. Induced submucosal edema. Level 3^5. HE, lens × 10.

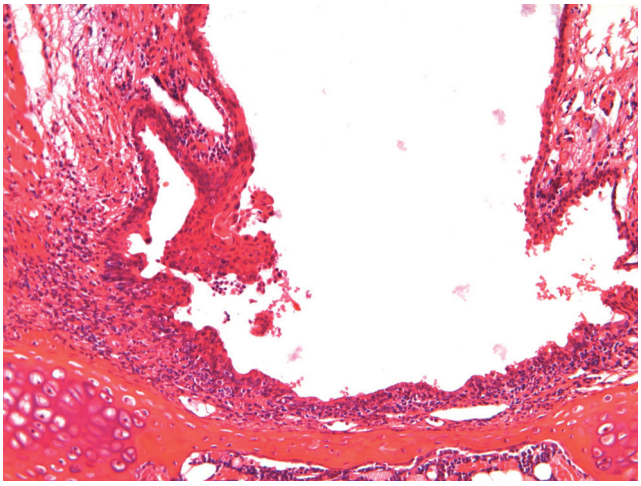

Fig. 14. Induced submucosal chronic active inflammation. Squamous metaplasia with focal hyperplasia and keratinization at arytenoid projections. Level 3^5. HE, lens × 20.

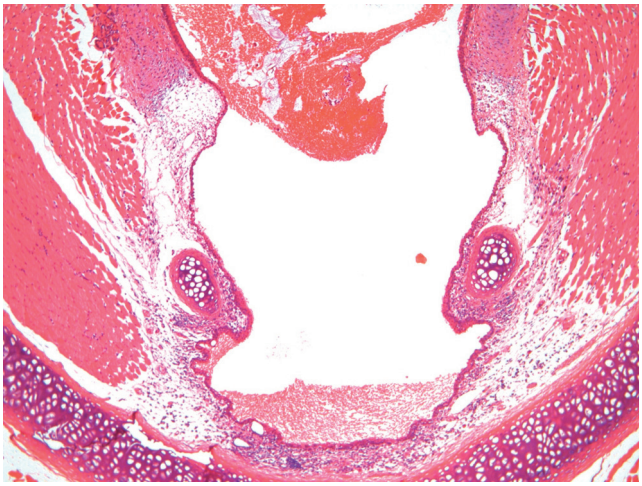

Fig. 12. Induced submucosal subacute inflammation. Level 3^5. HE, lens × 10.

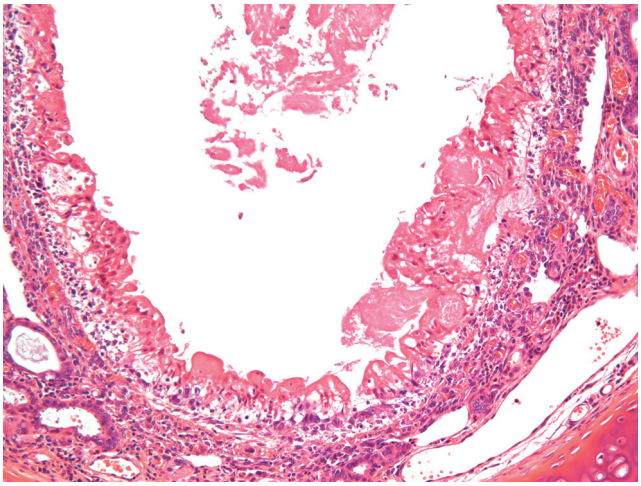

Fig. 15. Induced necrosis of respiratory epithelium. Level 2^5. HE, lens × 20.

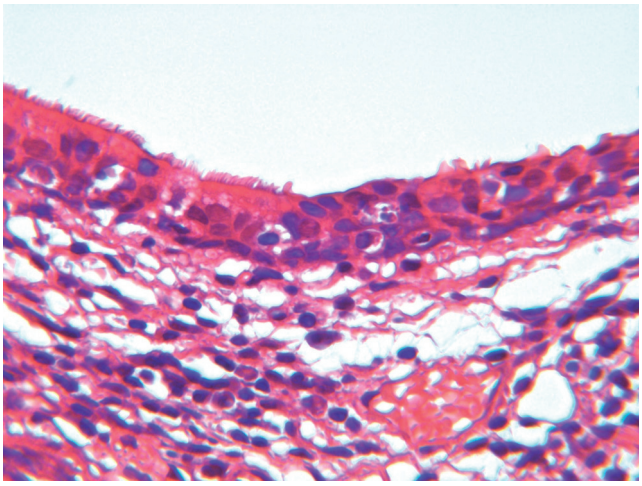

Fig. 13. Induced submucosal subacute inflammation. Partial loss of cilia from respiratory epithelium. Level 3^5. HE, lens × 100.

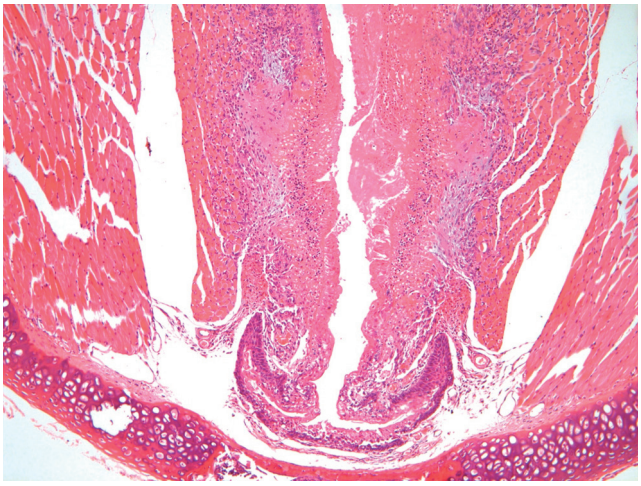

Fig. 16. Induced extensive necrosis of submucosal and mucosa. Level 2^5. HE, lens × 10.

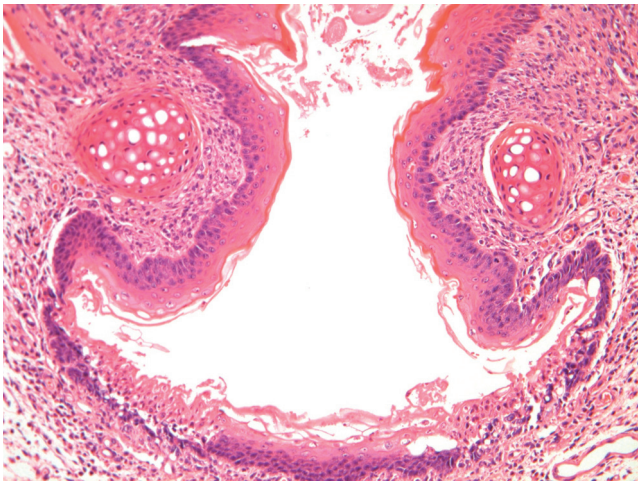

Fig. 17. Squamous metaplasia along with submucosal inflammation and mucosal necrosis. Level 3[5]. HE, lens × 20.

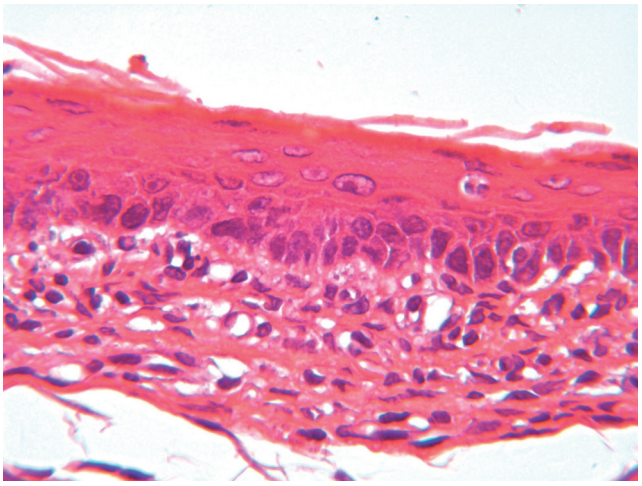

Fig. 18. Squamous metaplasia with keratinization. Submucosal inflammatory infiltrate. Level 6[5]. HE, lens × 100.

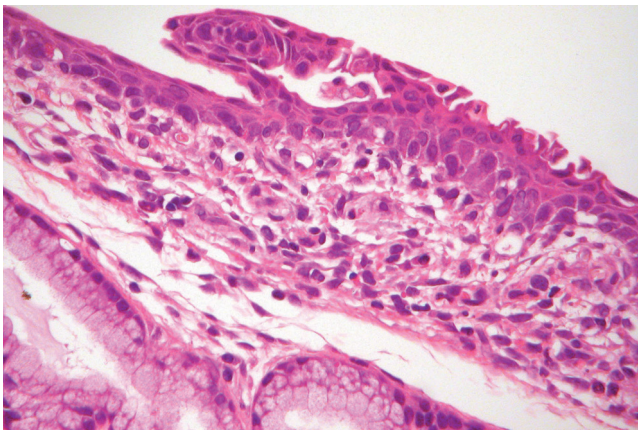

Fig. 19. Squamous metaplasia and focal hyperplasia. Submucosal inflammatory infiltrate. Horizontolongitudinal section. HE, lens × 100.

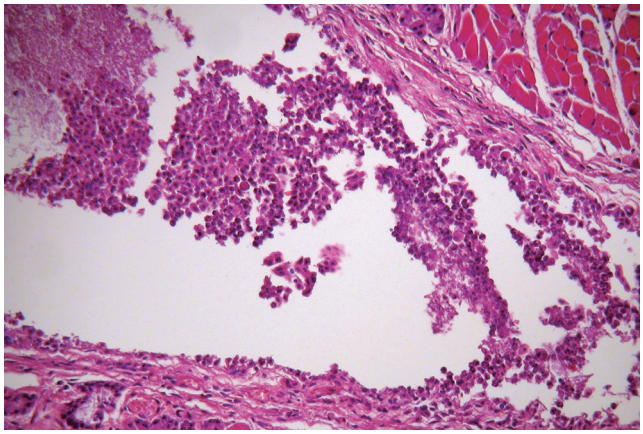

Fig. 20. Reflux laryngitis. Mucosal necrosis and inflammatory infiltrate and secretion. Horizontolongitudinal section. HE, lens × 10.

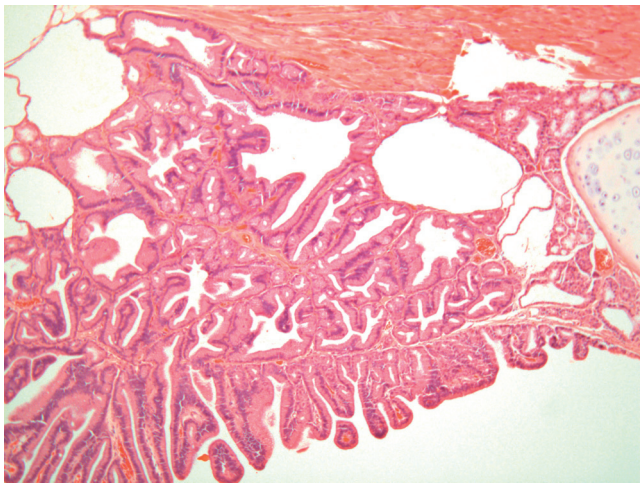

Fig. 21. Spontaneous adenomatoid lesion in control female Fischer 344 rat f (46 weeks at age) [43]. HE, lens × 20.

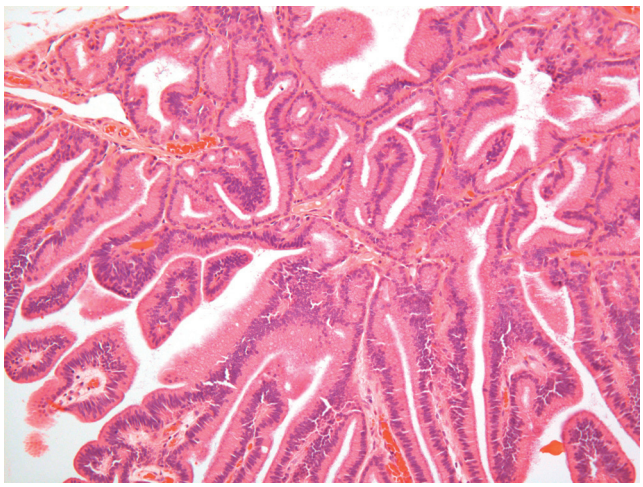

Fig. 22. Spontaneous adenomatoid lesion in control female Fischer 344 rat f (46 weeks at age) [43]. HE, lens × 40.

both sexes only at the high dose group at level 6, whereas squamous metaplasia was recorded in a high percentage of the mid and high dose animals at level 6. In a single case, squamous metaplasia was noted at the arytenoid projections at level 3. In the latter study (4-week study T7), subacute inflammation in levels 2, 5 and 6 along with multifocal necrosis in levels 3–6 were accompanied by multifocal squamous metaplasia (Fig. 17) and regenerative hyperplasia at levels 4–6, i.e. induced squamous epithelial changes were noted as a consequence of inflammatory lesions (necrosis, submucosal inflammation).

In another study (acute study T3), the metaplastic squamous epithelium was keratinized at level 6 (Fig. 18). Moreover, the lesion also included submucosal inflammation, dilation of submucosal sero-mucinous glands and submucosal fibrosis.

Squamous metaplasia was also a predominant finding in studies that were evaluated by horizontolongitudinal sections. However, in all cases it was accompanied by inflammatory processes, i.e. submucosal inflammation (Fig. 19) (14-day study L4) or by increased incidences of glandular dilation and mononuclear cell foci. Degeneration/necrosis of the respiratory epithelium and a partial replacement by hyperplastic squamous metaplasia were also observed (4-week study L9).

Other organs of the respiratory system may or may not be involved. In study 13-week T6, there were inflammatory lesions including fibrosis in the lungs and a deposition of the test item in the nasal mucosa without any indication of degeneration, as well as, a deposition of the test item in the tracheobronchial lymph nodes. In 13-week study T1, 26-week study T2 and 26-week study T5, there were changes in the Bowman's glands along with degenerative and regenerative changes in the olfactory mucosa of nasal cavities. There were increased incidences of hyaline inclusions in the nasal cavities as well as epithelial degeneration/disorganization in the olfactory mucosa. In 26-week study T1 and study T3, alveolar histiocytosis along with hemosiderin deposition was present. Interstitial inflammation of the lungs along with alveolar wall hyperplasia/hypertrophy was recorded in 4-week study T7. In the nasal cavities of 14-day study T2, hyaline inclusions were recorded in the olfactory mucosa, and in 14-day study L4, the laryngeal lesions occurred along with degeneration/disorganization of the olfactory mucosa accompanied by erosions and squamous metaplasia. Moderate necrotic changes were recorded in the nasal cavities in another 4-week study L9. In the remaining 41.2% of all studies with induced laryngeal lesions, no further findings were recorded in other respiratory tissues.

Spontaneous lesions recorded in non-inhalation studies

In non-inhalation studies, lesions were extremely rare and consisted only of a few cases with hemorrhage, one granuloma, one case of inflammation, and a few cases with impacted food. These studies were all evaluated by horizontolongitudinal sections.

In oncogenicity studies, laryngeal lesions were also rare. They consisted of single cases of mononuclear cell foci or inflammation, as well as, food impaction within the laryngeal lumen. Neoplastic changes were not observed in any study, however, there were metastases of malignant lymphoma, carcinoma and sarcoma each in single animals of 104-week study 3.

Induced lesions recorded in non-inhalation studies

Induced lesions were recorded only in 3 studies. Impacted food as a consequence of dysphagia due to salivary gland atrophy was recorded in the high dose group of a 26-week study (26-week study 4). This was also deemed to be the cause of death in the respective animals. Regurgitation laryngitis was recorded in 2 studies (14-day study 5 and 4-week study 18). In both studies, the lesions consisted of mucosal necrosis along with inflammation and inflammatory secretion (Fig. 20), In the 4-week study, the findings partially recovered after a 2-week treatment-free period. In both cases, the nasopharyngeal duct was heavily involved with necrosis, inflammation and squamous metaplasia, and in the nasal cavities including the maxillar sinuses a wide range of inflammatory and regenerative lesion were also recorded.

Discussion

Information on spontaneous and induced laryngeal lesions is limited in the published literature. They consist mainly of reports on induced lesions from inhalation studies. Repeated inhalation of test items induces a wide range of responses, depending on the physical properties of the toxicant and concentration and duration of exposure[4]. Specific areas of the rodent laryngeal mucosa appear to be more sensitive to inhaled materials and respond by injury. The exact knowledge of laryngeal histology is important for the detection of subtle changes. The rat larynx contains 5 epithelial types (stratified squamous, low squamoid, respiratory, and 2 forms of pseudostratified cuboidal epithelium) of which every epithelial type has a specific location[6]. Most sensitive regions in the larynx include the epithelium covering the base of the epiglottis, ventral pouch, and the medial surfaces of the vocal processes of the arytenoid cartilages[1].

Different trimming techniques were confirmed to be of major impact on incidences of spontaneous and induced lesions of the larynx. Horizontolongitudinal sections were considered to be of little value due to the fact that important structures are not met on the section plane. An interesting trimming technique was found to be the sagittolongitudinal sectioning which results in a very satisfying overview on the laryngeal structures in the complete course of the laryngeal length[7]. Furthermore, it was deemed to be very important that this technique provide information on the current contents in the laryngeal lumen that may be influenced by impacted food particles or even the test item. The latter

technique was helpful for mechanistic considerations in studies with a high number of decedents by asphyxia. Transverse techniques are superior due to cutting planes at sensitive structures[5,8]. The disadvantage is loosing connectivity of laryngeal tissues and the overview on the complete structures. Furthermore, such techniques are not advisable in certain studies, e.g. compounds leading to dysphagia (e.g. M3-inhibitors) may cause food impact in the laryngeal lumen causing death. In transversal sections, a bolus may be lost. The horizontolongitudinal sections however revealed mostly negative results due to the fact that sensitive regions are not usually included in the section plane. When inhalation studies were examined based on horizontolongitudinal sections, in 88.9% of all acute studies, 50% of all 14-day studies and 75.0% of all 4-week studies, there were no lesions recorded in the larynx. Similarly, in non-inhalation studies based on horizontolongitudinal sections, only 3 studies revealed lesions; in 2 of the studies there was regurgitation laryngitis and in the third study, there was food impaction by decreased salivation and dysphagia. In contrast, 100% of all studies of these durations evaluated by transversal sections had lesions reported.

In order to determine the influence of different vehicles, the impact of different application routes in toxicity studies, and differences between induced vs. spontaneous lesions; the authors compiled and reviewed the data obtained from a large number of studies performed at Harlan Laboratories Ltd. Itingen during 1997–2007. The comparison of lesions encountered in control animals of inhalation studies treated with different vehicles, i.e. air, lactose, Mg-stearate, and saline, did not reveal differences in the type, distribution pattern, incidence and/or severity of spontaneous lesions. The type of lesions was not different when studies with different application routes were compared in non-inhalation studies and inhalation studies. The incidence was dependent on different trimming techniques used for these study types.

There were minor gender- and age-related differences although the cause was not clear. They consisted of higher incidences of impacted food material/foreign bodies in males compared to females in acute to 13-week studies, but not thereafter. Desiccation of glandular secretion was noted at higher incidences in males from acute to 13-week studies along with inflammatory lesions in 14-day to 4-week studies. On the other hand, mineralization accompanying desiccated glandular secretion was more often recorded in females in 4- and 13-week studies. Spontaneous squamous metaplasia of the mucosa overlaying the ventral aspect of the larynx at level 6 became evident earliestin in 14-day studies with a higher incidence in females. This higher incidence in females was recorded in studies up to 13 weeks, and thereafter, squamous metaplasia at level 6 was recorded at higher incidences in males.

A strain-related high incidence of granulomatous lesions at the epiglottis (38%), inflammation at the basal part of the laryngotracheal junction (26%), cystoid necrosis and/or calcification of the laryngeal and/or tracheal cartilage (72%) and food impaction (24%) was described in Fischer 344 rats at 69 weeks of age. In 5 other strains (Sprague Dawley, Wistar, Brown Norway, Dark Agouti and Lewis) these lesions were rare. It was concluded that especially females exposed to chronic daily irritation by gavage, are predisposed to have a high mortality rate in chronic toxicologic tests due to the disposition to a high incidence of cartilage degeneration, which presumably leads to a dysfunction of the larynx[7]. Impacted food was found in most of the dead animals (53%) in the oropharyngeal cavity along with granulomatous inflammation of the sero-mucinous glands (60%), frequently along with papillary projections (50%) into the lumen[9]. The onset of these findings was also reported to be age-related. The onset of tracheal and laryngeal cartilage was recorded as early as 6 weeks of age. Granuloma in the oropharyngeal region or laryngeal cartilage and chondroid degeneration or osseous metaplasia were recorded at high severity degrees already at 19 weeks of age[10]. These observations lead to the consideration of age-related laryngeal dysfunction/dysphagia by degenerative and inflammatory processes in Fischer 344 rats.

Age-related lesions may be also related to impairments in age-related laryngeal kinematics in rats leading to discoordination of laryngeal and respiratory movements causing age-related swallowing and communication impairment. It was demonstrated, that in young animals, glottal opening began before the onset of inspiration, and glottal and respiratory cycles were phasic and stereotypic. In contrast, in old animals, inspiration often began during the glottal closing phase, and both respiratory signals were asymmetric[11]. This is supported by the observation, that aging was associated in F344/N rats (comparison of animals at 12, 24 and 35 months of age) with the reduction of laryngeal sensory and secretomotor nerve endings. The large sized motor end-plates localized in thyroarytenoid and cricoarytenoid muscles were degenerated in aged rats, whilst the small sized motor end-plates, localized predominantly in vocal muscles, did not show any age-related changes[12]. Other indicators for degenerative changes related to probably dysfunction of the larynx were reported to consist of abundant mitochondrial clusters and ragged red fibers in the muscles of 30 months old Fischer 344 × Brown Norway F1 hybrid rats, and there was an age-related increase in glycogen-positive fibers leading to weaker, slower, and more fatigable thyroarytenoid muscles with age[13]. In patients, significant differences between the young adult larynx and the geriatric larynx were found for vocal fold bowing, prominence of the vocal process, glottic proportion, phase and amplitude symmetry of mucosal wave and tremor of laryngeal structures[14].

The pattern of spontaneous lesions in the rodent larynx was found to be dominated by degenerative and inflammatory lesions starting most often in the submucosal glands at posterior regions (levels 2 and 3 by Sagartz[5]) by dissecated secretion followed by mineralization. These processes are considered to be involved in causing glandular inflammation at the posterior levels. In several cases,

impacted foreign bodies, mainly food remnants and rarely hairs, induced inflammatory lesions.

It is concluded, that in rodents induced lesions of the larynx differ in type, distribution pattern, severity and incidence from spontaneous lesions. Similar induced lesions as described under 'Results' have been reported: acute necrosis and inflammation (Dodecanediamine[15]); acute inflammation and hyperplasia of the respiratory epithelium (CS2 (94% o-chlorobenzalmalononitrile [CS]; 5% Cab-O-Sil(R). colloidal silica; 1% hexamethyldisilizane)[16]); suppurative inflammation (bromoethane[17]); inflammation (necrotizing, chronic, or suppurative) (Hexachloro-cyclopentadiene[18]); chronic inflammatory infiltrate (calcium carbonate[19]); hyperplastic and metaplastic epithelial changes (cigarette mainstream smoke[20]); squamous metaplasia (Hexachlorocyclopentadiene[18]); hyperplasia and squamous metaplasia in the free edge of the vocal fold and squamous hyperplasia on the middle portion of the vocal fold (cigarette smoke[21]); keratinizing metaplasia (cigarette smoke[21]); minimal squamous epithelial hyperplasia and degeneration/ necrosis of the cartilage (1,3-dimethyl-2-piperidinone and 1,5-dimethyl-2-piperidinone[22]); squamous metaplasia (Ozone (CAS No. 10028-15-6) and Ozone/NNK (CAS No. 10028-15-6/ 64091-91-4)[23]);and disturbed stratification of epithelium covering the true vocal cords shows (mosquito coils[24]). Some cells of squamous metaplastic epithelium were reported to exhibit loss of desmosomal connections between cells and enlargement of the intercellular space (cigarette smoke[25]).

Induced lesions in non-inhalation studies were found to be exclusively related to reflux laryngitis and food impaction.

Reflux laryngitis was observed rarely in the compiled studies (2 studies only: (14-day and 4-week gavage study). Reflux laryngitis may however affect the study outcome by morbidity and death. It is also a clinical issue in patients, whereby inconsistencies in histological patterns makes the diagnose difficult[26]. Different rat models investigating impacting factors on reflux laryngitis to mimic the human conditions were developed as there is the determination of the relationship between reflux and laryngeal hypersensitivity and the influence of pepsin[27] or the influence of bile reflux[28]. Also, there have been discussions about the possible relationship of reflux laryngitis and laryngeal cancer. Inpatients with reflux laryngitis posterior laryngitis, inflammatory polyp of the larynx, ulcer or laryngeal granuloma, subglottic stenosis and laryngeal squamous cell carcinoma has been reported[29]. In rat models reflux did not appear to be an independent risk factor for laryngeal carcinogenesis, but was considered to enhance the etiologic risk factors[30].

Other laryngeal lesions recorded consisted of rare epithelial disorganization adjacent to the metaplastic squamous epithelium layer. Induced inflammatory lesions varied and included focal or multifocal glandular dilation in posterior levels, submucosal edema with scattered inflammatory cells, mainly granulocytes and plasma cells and/or submucosal acute to subacute inflammation, squamous metaplasia with hyperplasia and squamous metaplasia or keratinization at the arytenoid projections, erosions or necrosis of large portions of the lining epithelium without preference of an epithelial type and regenerative hyperplasia. In several cases, squamous metaplasia was accompanied by with submucosal inflammation, dilation of submucosal sero-mucinous glands and submucosal fibrosis. The rapid onset of recovery as found in the examined studies is not surprising. Time-course of repair was described in a rat model of vocal fold scarring. Scarred vocal folds showed less hyaluronic acid and more collagen types I and III than did controls. Type III was stable for 12 weeks, while type I declined until 8 weeks and thereafter remained unchanged. Fibronectin increased for 4 weeks and then decreased. In summary, tissue remodeling process in scarred vocal folds was approximately 2 months after wounding[31]. In Sprague-Dawley rats, re-epithelialization occurred by day 3 and was complete by day 14 after wounding. Moreover, it was shown that granulation tissue was formed by day 3. Hyaluronic acid and collagen type I appeared in injured vocal folds by day 3, peaked at day 5, and thereafter decreased. Collagen type III and fibronectin appeared by day 1 and continued to be intense at all time points after day 3[32].

The squamous metaplasia of the respiratory epithelium overlaying the ventral gland in level 6 is the most common induced lesion in rodents. This epithelial change in the larynx of rodents is an issue of frequent discussions with respect to its biological and toxicological significance. In level 6 according to Sagartz[5], spontaneously existing squamous metaplasia was recorded as a spontaneous lesion only in male Wistar rats from 13-week inhalation studies and in longer lasting studies. Also, the most common induced lesion consisted of squamous metaplasia of the originally ventral respiratory epithelium overlaying the ventral gland does not differ from the spontaneous occurring squamous metaplasia. In an oncogenicity study, the incidence was at 20.0% in control animals without any indication of dysplasia or hyperplasia. The lesion is considered to be indicative of the especially high sensitivity of the rodent larynx to any mild irritant and cannot be regarded to be of adverse nature as long it occurs focally at this location and is low in degree[45]. According to the results of a recent ESTP international expert workshop[46], the workshop experts found that many induced or spontaneous minimal laryngeal lesions reported in inhalation exposure studies does not fullfil the criteria of a completed "squamous metaplasia" (WHO nomenclature citation). These minimal, focal epithelial changes of the larynx epithelium predominantly occurring at the base of the epiglottis should be given the descriptive term of an "epithelial alteration" and assessed as "non-adverse". In addition, cases of minimal to slight, focal "laryngeal squamous metaplasia" that are not observed diffusely are assessed as "non-adverse" as well. They are not considered precancerous lesions. "Squamous metaplasia" was reversible in nature with all compounds examined and reported in this paper. However, granulomatous

inflammation leading to the formation of polypoid lesions extending into the lumen and potentially decreasing airflow was reported[33]. Progression to laryngeal neoplasms was reported rarely in hamsters but not in rats[34–42] and are summarized elsewhere[32].

It was reversible in nature with all compounds, which were examined. However, granulomatous inflammation leading to the formation of polypoid lesions extending into the lumen and potentially decreasing airflow has beenreported[33].

Spontaneous neoplasia in the rat larynx is an extremely rare event. A spontaneous adenomatoid lesion of a female control Fischer 344 rat (46 weeks at age) has been reported[43] (Fig.21,22). Furthermore, a single polyp, granular cell tumor and papilloma have been published in Fischer 344 rats[44].

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