Introduction
Toxicity studies by intravitreal administration (in the eyes) are now frequently performed in rabbits and minipigs to assess the non-clinical safety of pharmaceuticals which are intended to be administered to human patients by this route. The present poster describes and illustrates the most frequent morphological findings encountered in these studies. It is important to interpret and understand these findings, in particular, it is critical to differentiate spontaneous or injection-related findings from treatment-related effects.

Materials and Methods
Histology sections of the eyes from 282 New Zealand White rabbits, 148 Dutch Belted rabbits and 227 Göttingen minipigs, obtained during toxicity studies conducted by the intravitreal route, were reviewed.

All eyes were fixed in modified Davidson’s fixative for a maximum of 96 hours. Then they were rinsed and preserved in 10% formalin. Histological examinations were conducted on sagittal and two parasagittal sections stained with hematoxylin and eosin (in total, 2680 eye sections from rabbits and 1362 eye sections from minipigs). The lens was examined in situ.

As the eye is a very complex organ with numerous structures, each of its compartments was histologically examined, and lesions were reported using descriptive terminology. For this purpose, a predefined descriptive microscopic glossary, including general diagnoses (e.g. findings with names terminating in ‘itis’), was built up by a working group of seven CIT pathologists to ensure inter/intraday homogeneity and historical data availability.

Results and discussion
The microscopic findings were similar in all species/strains and were categorized as spontaneous, injection-related or treatment-related.

Normal anatomical or artefactual features
Some normal features observed in rabbits needed to be differentiated from lesions. These included:
- Depression of the optic disc; which should not be misinterpreted as a secondary sign of intraocular pressure increase.
- Loose stroma in the iris and/or ciliary bodies-processes; which could be seen in untreated control rabbits and should not be misinterpreted as edema.

Artefactual changes associated with sampling, trimming and slide processing may modify the histological appearance of several structures. These included:
- Focal damage of the retina/choroid (which is differentiated from true erosion/ulceration by the absence of inflammatory cell infiltrates).
- Swelling in the corneal stroma (to be differentiated from edema).
- Vacuolation in the lens (which usually appears as clear vacuoles differing from the irregular variable sized eosinophilic/droplets observed in lens degeneration).
- Retinal separation. This artefactual artifact where the retina is separated from the choroid, should be differentiated from retinal detachment on the basis of lack of other associated retinal lesions.
- vacuolation (rare).

Spontaneous findings
Very few spontaneous lesions were seen in rabbits, and these consisted of occasional small isolated clusters of enlarged cells (probably hypertrophied retinal pigmented cells) in the subretinal space (Image 2). These were occasionally observed in New Zealand White rabbits with a similar incidence and severity in control and treated animals (the incidence of affected animals varied from 0 to 80% between studies).

No spontaneous lesions were observed in the minipig.

Injection-related findings:
Frequent minor focal changes attributed to the injection procedure were observed on the bulbar conjunctiva. Most rabbits and minipigs were affected. Depending on the number of injections, and the time lapse between injections and necropsy, these changes consisted of:
- Focal acute inflammation (e.g. homoeopathy/edema),
- Subacute or chronic inflammation (e.g. granulocytes/heterophils, and/or mononuclear cells).

Focal damage of the retina/choroid was occasionally observed at the injection site near the ora ciliaris retinae. The incidence of affected animals varied from 0 to 80% between studies.

Most treatment-related lesions observed in rabbits and minipigs consisted of slight to severe acute to chronic inflammatory and/or degenerative changes. These lesions involved the different structures of the eye and included:
- Vitreous body:
  - haptic/ciliary bodies;
  - Occasional mononuclear inflammatory cell infiltrates in the iris or ciliary bodies. This usually correlated well with signs of uveitis at the ophthalmological examination.
- Optic nerve:
  - Increased cellularity (Image 3): rarely observed and when present, usually associated with severe inflammatory changes in the ocular globe.
  - Vascularization (rare).
- Lesions related to increased intra-ocular pressure: Features indicative of increased intra-ocular pressure included enlargement of the eyeball (buphthalmia), thinning of the cornea with erosion/ulceration, atrophy of the retina, especially in the ventral portion, and excavation (cupping) of the optic disc (Image 8).

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The most common ocular histology lesions observed in rabbits and minipigs after intravitreal injection during toxicity studies

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Conclusion and discussion
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AC: anterior chamber; CB: ciliary bodies; CH: choroid; L: lens; OD: optic disc; ON: optic nerve; R: retina; S: sclera; nuc: nucleus; st: stroma; TR: trabecular meshwork; V: vitreous body.

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