Notes and miscellanea

Effects of bis(tri-n-butyltin) oxide on the eyes of rabbits

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Bis(tri-n-butyltin) oxide and its various commercial preparations are widely used because of their bactericidal, fungicidal, algicidal, and insecticidal properties (Barnes and Stoner, 1958; Barnes and Stoner, 1959; Elsea and Paynter, 1958; van der Kerk and Luijten, 1954). The commercial preparations of TBTO are used in industry and in agriculture and as disinfectants in hygiene services (Ascher and Nissim, 1964; Daum, 1965; Grün and Fricker, 1963; Hedges, 1957; Hudson, Sanger, and Sproul, 1959; Stecker, 1957).

With the growing use of TBTO and its preparations an increasing number of people are coming into contact with them with the risk of injury, particularly to exposed tissues, such as the skin and eyes.

In our experiments we used TBTO as the commercial product Lastanox, which is identical with many other similar products having TBTO as their basis, such as Hollicide, bioMET, TBTO, FungiBan, Tin anti-slime, etc. All these products are similar in their active component (TBTO) and therefore in their effects and use, the only differences being in the vehicle used and in the TBTO content. The only effective substance contained in Lastanox is TBTO.

Because only very few experimental studies on the harmful effects of TBTO on individual tissues have been published — and, to our knowledge, none has been published on the eyes — we decided to investigate the effects of TBTO on the eyes of rabbits.

Methods

TBTO was applied using two forms of Lastanox: 1 Lastanox 'T': 20% TBTO in lower alcohols and non-ionic surface active substances (n-alkyl-polyethylene oxide), and water. 2 Lastanox 'P': 15% TBTO in the same vehicle with the addition of bis(5-chloro-2-hydroxyphenyl)methane.

Albino rabbits, male and female (1:1) of the same age (10 months), average weight 800 to 900 g., were divided into four experimental and three control groups. Each experimental group contained six animals, each control group four animals. All the rabbits received 'standard' diet and were observed three times a day over a period of 100 days.

Experimental groups

Lastanox 'T' and 'P', diluted with water, were used in concentrations of 10 and 1%. All rabbits in each experimental group received identical Lastanox ('T' or 'P') in the same concentration. A single dose of 0·03 ml. per head was applied in drops into the conjunctival sac of the left eye, once only, on the first day of the experiment. The actual doses of TBTO in mg./kg. body weight of rabbits in the different experimental groups were: group I, 10% Lastanox 'T' = 6·1 mg./kg.; group II, 10% Lastanox 'P' = 4·6 mg./kg.; group III, 1% Lastanox 'T' = 0·61 mg./kg.; group IV, 1% Lastanox 'P' = 0·46 mg./kg.

Control groups

The rabbits in the control groups received by the same method a dose of 0·03 ml. per head of the following undiluted solutions: group V, vehicle of Lastanox 'T'; group VI, vehicle of Lastanox 'P'; group VII, water.

Results

General clinical findings

All the animals in the experimental groups were weak and showed hyperreflexia on the second to fifth days after application. These signs had dis-
appeared spontaneously by the sixth day. Special clinical findings in two rabbits are described below.

No animal in the control groups showed any deviation from the normal clinical picture.

**Clinical findings in the eye and adnexa**

The changes caused by similar concentrations of the two Lastanox preparations ('\( T'\) and '\( P'\)) were nearly identical and will therefore be described together.

**Groups III and IV** One to three minutes after the application there was marked hyperaemia of the bulbar and palpebral conjunctivae accompanied by violent watering, miosis, and distinct blepharospasm.

Within 3 hours we observed erythema and mild oedema of the eyelids; numerous large necroses, petechial haemorrhages, and early chemosis of the bulbar and palpebral conjunctivae; and distinct pericorneal injection and dullness of the shine of the cornea with decreased transparency.

Within 12 hours the corneal transparency had decreased even further, the aqueous humour was opalescent, and the iris was oedematous and discoloured.

After 24 hours, the skin of the eyelids was markedly oedematous and erythematous and, besides the haemorrhages, point-like necroses and papules, about 1 mm. in diameter, could be seen. The palpebral fissure was narrowed, partly by the eyelid oedema and partly by the prolapse of chemotic conjunctiva, which also partly covered the limbus corneae. The conjunctiva showed extensive necrotic areas, 1 to 10 mm.\(^2\) in size, with signs of haemorrhage and suffusion in between. In all animals there was marked secretion which was mildly purulent in three cases. In the majority of animals marked episceral injection was noticeable.

The surface of the cornea was uneven, and its transparency was greatly decreased and showed numerous small whitish and vaguely demarcated infiltrations. The aqueous humour became turbid. The pupil was miotic, not properly rounded, and did not react to light. The iris was discoloured and the pattern indistinct. The red reflex could not be seen and the optical background and retina could not be examined.

Within 2 to 5 days of application, eschars and ulcerations, some 10 to 15 mm.\(^2\) in size, formed on the eyelids partly covered with pus. Between these eschars were numerous papules, pustules, haemorrhages, and small necroses. The necrotic conjunctiva was peeling off, leaving a bleeding underlying surface. Besides the infiltrations already mentioned, ulcerating surface erosions, sometimes three to six in one animal, appeared on the cornea. The ulcers had sharp borders and a dirty whitish base, and some were 5 mm.\(^2\) in size. The pupil was miotic, not rounded, and showed no reflex. The iris was discoloured in all animals and the pattern was absent. The red reflex could not be seen.

**Groups I and II** In these groups, the changes observed were similar to those noted in the foregoing groups, but they were considerably more pronounced both quantitatively and qualitatively, particularly the corneal ulcers, which developed sooner and in two cases perforated (later they closed and healed). Except in one rabbit, total opacity of the cornea developed together with a pronounced symblepharon in most cases. Also deformation of the eyelids was more marked, and in a few cases one eyelid became stiff and fixed.

**Pathological findings**

In one rabbit of both groups I and II the general clinical state markedly deteriorated 3 to 4 days after the application. The animals showed extreme weakness, their eyes were closed, and their heads hung down. On day 8 to 9 there was paresis of all limbs and on day 10 periodic respiration developed in both.

The rabbit from group I showed numerous suffusions in the bulbar conjunctiva, a massive congestion of the cornea, iridal haemorrhages, and disappearance of the anterior chamber. The rabbit from group II showed partial congestion of the cornea with hypopyon and corneal perforation in one of the ulcers. Further signs resembled those in the other rabbits.

Both the rabbits died suddenly, one on the eleventh day and the other on the twelfth day after application. At post-mortem the organs were fixed in 10\% formalin and histological sections were stained with haematoxylin and eosin.

**Macroscopic findings** The skin of the eyelids and adjoining the eye was necrotic, with deep-set defects having sharp borders, the bases of which were covered with purulent exudate. Large pieces of necrotic conjunctiva were peeling off and the cornea was opaque.

The brain, medulla oblongata, and all the abdominal organs were hyperaemic. No other macroscopic changes were found.

**Microscopic findings** The cornea was necrotic and full of neutrophils. The sclera was oedematous. There were foci of young granulation tissue. On the border of the sclera the necrotic surface showed patches of loose epithelium which covered the surface with one layer of cells. The iris was very congested, the capillaries near the surface of the eye were hyperplastic, the endothelium was markedly oedematous, and the lens was artificially dislocated. The retina was not affected.

At the sites of maximum damage, the skin
FIG. 1. Day 1. 0-61 mg. TBTO/kg. Lastanox 'T' 1%.

FIG. 2. Day 14. 0-61 mg. TBTO/kg. Lastanox 'T' 1%.

FIG. 3. Day 36. 0-61 mg. TBTO/kg. Lastanox 'T' 1%.

FIG. 4. Day 100. 0-61 mg. TBTO/kg. Lastanox 'T' 1%.

FIG. 5. Control. Day 1. Vehicle only of Lastanox 'T'.

FIG. 6. Control. Day 36. Vehicle only of Lastanox 'T'.
adjoining the eye was necrotic through its full depth to the subcutaneous layer and was permeated with numerous leucocytes, some of which had disintegrated. In places where there was less damage there were intra- and sub-epidermal pustules. Nearby some capillaries were thrombosed and in some places small foci of granulation tissue were found. The least affected areas showed segments with pustular changes and segments of non-purulent infiltration in the upper corium.

The spleen showed hyperplasia of the reticulo-endothelial cells. No histological changes were found in other organs.

Control groups
In the rabbits of all the control groups no abnormal macroscopic or microscopic changes were seen.

Discussion
As the materials tested in the control groups had no effect on the tissues of the eyes, it must be concluded that all the observed changes were caused only by TBTO. So far we have found only one report of the harmful effects of tributyltin compounds on the eye (Lyle, 1958). Lyle, however, did not state what doses and concentrations of TBTO were used.

The application of a single drop of TBTO into the conjunctival sac was chosen as being the typical mechanism by which most cases of accidental damage to the eye are caused.

As we assumed a high effectiveness of TBTO, we chose very low doses of this compound so that we could follow the minor changes that occurred in the eye. The results of our experiment confirm our assumption and show that even our theoretically chosen dose of TBTO represented the maximum that could be used without causing a rapid corneal perforation with all its inevitable complications. The histological findings corroborate the clinical observations.

We have found no previous report of experimental damage to the eye, caused by TBTO, in any species of animal. Although we accept that there are differences between the human and the animal eye and also that there are differences connected with environment, nevertheless we believe that our results form a useful contribution to the study of the harmful effects of TBTO on the human eye.

References

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