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Statement

From the Committee for Anaesthesia and Analgesia

On the use of tribromoethanol (TBE, Avertin®, E107, Renarcol®, Byk 250) in laboratory animals

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Historical background and field of use

The use of tribromoethanol (TBE, Avertin®, E107, Renarcol®, Byk 250) for anaesthesia dates back to the early part of the last century, when few anaesthetics were available for humans and animals. The substance was described by Willstater and Duisberg in 1923, and Fritz Eichholtz studied its anaesthetic effect in animals (e.g., according to Veal 1931). Surgeon Otto Butzengeiger introduced Avertin® in 1926 for clinical use in humans, instilling the solution rectally. While Avertin® was mainly administered as a so-called rectal anaesthesia, it was also administered intravenously (Lundy 1929). It was described as a basal narcotic (Lundy 1929, Eichholtz 1930), because it led to gentle induction, which was supplemented mainly with ether because of the relatively duration of action or in the event of insufficient depth of anaesthesia after loss of consciousness was reached (Schildbach 1930, Behrend 1931).

The use of Avertin® was contentious (Killian 1926, Lundy 1929, Goerig and Schulte am Esch 2003) from the outset, to begin with primarily because of its respiratory depressant properties (Kotzoglu 1929, Ranft and Kochs 2004). Then increasing reports of high mortality rates soon began to appear, in which a direct toxicity of the anaesthetic was assumed (Killian 1926, Kotzoglu 1929, Lundy 1929, Eichholtz 1930, Veal et al.1931). During the early years, in particular, a further side effect was observed in the form of an inflammation of the intestinal mucosa, which generally healed after 2-6 days, but also led to death in some cases, for example as a result of diarrhoea, intestinal atony, ileus and intestinal perforation. The cause of the local reaction was attributed to a degradation of the Avertin® solution (Killian 1927, Kotzoglu 1929, Eichholtz 1930). With increasing experience in the production and handling of Avertin® and also in dosing and in the preparation for possible side effects, rectal induction of anaesthesia with Avertin® became safer (Behrend 1931). Avertin® was quickly driven out of the market as an injection anaesthetic by the short-acting hexobarbital, which was introduced in 1933, because this product had a much more persuasive clinical profile of action (Ranft and Kochs 2004). From the late 1940s onwards, there was a sharp decline in its use (Meyer and Fish 2005), but it was still occasionally used in children up until the 1960s. In the meantime, Avertin® can be assigned to the archives in the history of medicine (Ranft and Kochs 2004).

In veterinary medicine, Avertin® or TBE was used in cats, dogs, and other mammalian species, as well as reptiles and birds, by rectal and oral administration (Meyer 2005). It was administered orally, in some cases with the accustomed feed, to catch, restrain and anaesthetize wild animals (wild turkey, opossum, marmot, squirrel, snake and tortoise) (Lumb and Jones 1973a). In the early 1970s, Avertin® was still mentioned in textbooks on veterinary medicine, where an induction time of 2 to 3 minutes, the maximum effect after 15 minutes and a duration of anaesthesia of 2 hours are specified for oral and rectal use in dog and cat. Toxic effects and a mortality rate of 2% in the cat are mentioned, but no further details are given on the quality and depth of the anaesthesia (Lumb and Jones 1973b).

There are reports on parenteral administration in rats, guinea pigs and rabbits (Nicol et al. 1965) and also in Gerbils (Norris and Turner 1983). At present, TBE is used for intraperitoneal administration in biomedical research projects with rodents. It is in widespread use as a mono-anaesthetic for surgical procedures in generating genetically modified mouse lines, e.g. for embryo transfer and vasectomy (Mann 1993, Hogan et al. 1994, Fish 1997, Wixson and Smiler 1997, Weiss and Zimmermann 1999, Nagy et al. 2003). TBE is also used occasionally for

anaesthesia, restraint or sedation in various rodent models, e.g. for cardiological studies in mice (Patel et al. 2005, Lin et al. 2007), especially for echocardiography (Huang and Linask 1998, Hart et al. 2001, Kiatchoosakun et al 2001, Schaefer et al 2005, Chu et al. 2006). In addition, TBE is also used in rats (Gopalan et al. 2005).

Also with parenteral administration in animals, attention was already drawn in early publications to problems and side effects (Nicol et al. 1965, Tarin and Sturdee 1972, Green 1975, Mann 1993), which were similarly observed in later years. Over the decades, this resulted in a number of publications with conflicting results and conclusions. Against the background of good long-term experience from clinical use in many laboratories, reports of targeted studies repeatedly give rise to heated discussions to this day (Silverman 2003).

Preparation, storage, and properties of injection solution

Since Avertin® and Renarcol® are no longer commercially available, an injectable solution of the anaesthetic has for some years had to be prepared extemporaneously the by users from the pure chemical substance tribromoethanol. Consequently, the injected solution is not a proprietary medicinal product, but a pharmacologically and toxicologically untested solution.

The starting material, tribromoethanol powder, is supplied in differing degrees of purity (Lieggi et al. 2005a). For the preparation of the solutions, there is a wide variety of protocols which differ in the composition both of the stock solution and of the working solution. The injected volume and concentrations may likewise differ (Kuhlmann 2004, Lieggi et al. 2005a). A microbial contamination of the solutions – which is conceivable with this procedure – should be eliminated by sterile filtration before injection into the animal (Weiss and Zimmermann 1999).

It was recognized early on that Avertin®, or TBE in solution, does not remain stable indefinitely. It was therefore recommended that the working solution be tested for pH and toxic degradation products before injection, e.g. using the Congo red reaction, detection of bromide with silver nitrate solution and other simple methods (Killian 1926, Lundy 1929, Schildbach 1930, Nicol et al. 1965). It has also long been known that the stock solution and the working solution should be stored at +4°C and protected from light (Papaioannou and Fox 1993, Hogan et al. 1994, Weiss and Zimmermann 1999, Nagy et al. 2003). But opinions are divided on the stability of the working solution: while a shelf life of several months is considered reasonable by some (Nagy 2003), others advise preparing the working solution afresh for each working day (Weiss and Zimmermann 1999).

The lack of standardization in the composition, preparation, and storage of the TBE solution and possible lack of care were frequently regarded as the cause of fatal side effects (peritonitis, high mortality, see below).

An attempt was therefore recently made to draw up guidelines for the preparation, storage and use of TBE. A two-part study on various was carried out for this purpose. The aspects first studied, using modern analytical methods, were the following:

1. Purity of the undissolved TBE powder from three commercial producers,

2. Degradation products (dibromoacetaldehyde) in working solutions prepared according to nine different, published protocols,
3. Degradation products and changes in pH under four different storage conditions for stock solution and working solution,
4. Testing of a stock solution and a working solution that unintentionally caused deaths.

Relevant differences were found in the purity or contamination of the TBE powder. None of the nine protocols for the preparation of a working solution differ in terms of degradation. Only with storage at +4°C and in the dark did the pH remain stable. A correctly stored and prepared stock solution and working solution caused high mortality but did not show any changes in the pH value or the degradation product dibromoacetaldehyde. These solutions revealed a substance (in 1H nuclear magnetic resonance spectra) that was not precisely characterized, indicating that a contamination or degradation could already have occurred during storage of the TBE powder by the producer or distributor (Lieggi et al 2005a, Meyer and Fish 2005).

More recent studies arrived at the following conclusions.

The injection solutions may contain toxic substances, although only some of them have been identified. Analysis of the injection solution using simple chemical methods does not seem capable of providing reliable indications of the toxic potential. It is not currently proven whether toxic compounds are present in the starting material (TBE powder) as a result of contamination and/or chemical processes during storage, but the possibility cannot be ruled out. Thus, it is still largely unclear how components with a toxic effect get into the injection and how this can be reliably prevented.

The problems with the standardization of the injection solutions are well-known. It was therefore suggested that testing be carried out on each new preparation of the stock solution, titrating the dose based on the depth of the anaesthesia in a number of mice and monitoring the general condition and survival of the animals for 3-4 days after intraperitoneal injection (Hogan et al. 1994, Nagy et al. 2003). The criticism of this procedure (Kuhlmann 2004) should not be dismissed out of hand, because it not only involves an increase in the number of animals used, but also accepts that the animals could possibly die a painful death.

Characterization of the anaesthetic

Properties and side effects of anaesthesia

The intraperitoneal injection of TBE has been described as a reliable method for the rapid induction of anaesthesia with surgical tolerance and a short recovery time in mice (Papaioannou and Fox1993, Wixson and Smiler 1997), Gerbils (Norris and Turner 1983) and rats (Gopalan et al. 2005). A relatively short anaesthesia is to be expected: in ICR mice, the period of the surgical tolerance stage has been reported to be 4-15 minutes (mean 7 min; Gardner et al. 1995) and 18.5 minutes (Lieggi et al. 2005b); in NMRI mice, 20-30 minutes have been reported (Weiss and Zimmermann 1999). More precise scientific studies on the properties of TBE as a mono-anaesthetic for surgical procedures in rodents do not appear to be available.

In 1972, Tarin and Sturdee reported on pathological changes in the abdomen after intraperitoneal injection of TBE in mice, in which ileus led to a mortality of 40% (Tarin and Sturdee 1972). Intestinal complications in mice were confirmed by Green (Green 1975) and also observed by Norris and Turner in Gerbils (Norris and Turner 1983). Fibrous adhesions in the abdomen, peritonitis, fibrosis of the liver capsule and ileus were reported in rats, which also showed clinical symptoms such as apathy, dehydration, an arched back and porphyrin discoloration (Reid et al. 1999).

In contrast, Papaioannou and Fox reported a mortality of <1% in a 2.5-year, retrospective study with more than 300 mice. The histopathological examination of abdominal tissue did not show any significant lesions in 10 suckling mothers following necropsy one week after repeat injection of TBE. A further 29 mice were studied for 2 weeks to 10 months after a first injection of TBE (Papaioannou and Fox 1993).

Weiss and Zimmermann also found no cases of death in more than 5000 mice anaesthetized with intraperitoneal injection of TBE over the course of 10 years. Necropsy did not reveal any pathological findings in 250 animals, although the time of injection is not apparent (Weiss and Zimmermann 1999).

Zeller and co-authors studied 30 suckling mothers 24 hours after injection of TBE and found necrotic lesions in the abdominal wall muscle and on the surface of the abdominal organs, as well as acute inflammatory signs in the peritoneum and fibrinous serositis of the abdominal organs in 28 animals. The extent of the lesions was dependent on the concentration of the injection solution: a 1.2% solution induced weaker signs of inflammation than a 2.5% solution (Zeller et al. 1998). Yet the influence of non-standardized preparation conditions of the TBE injection solution on the extent of the toxic effect could not be ruled out in every aspect (sterile filtration; Weiss and Zimmermann 1999, Zeller et al. 1999).

Pathological lesions comparable with the findings by Zeller and co-authors were likewise observed by Lieggi and co-authors 24 hours after injection, with marked decreases in inflammation after 4 and 10 days (Lieggi et al. 2005).

In the second part of the above-mentioned study aimed at drawing up guidelines for the preparation, storage and use of TBE, various series of experiments were carried out with ICR mice. It was shown that a TBE working solution with a low pH and stored in light and at room temperature also had a good anaesthetic effect and did not differ in this respect or in the induction of pathological lesions in the abdomen from a TBE solution that had been prepared and stored under optimum conditions.

It was found that, even under optimum conditions and the strictest adherence to working processes, the effects and side effects of anaesthesia with TBE were not reproducible. For example, with identical starting substances and protocol, the duration of surgical tolerance in three series varied from 18 to 37 and 46 minutes. In the last series, 17 mice were injected with freshly prepared TBE stock and working solutions of a TBE powder of the highest possible purity. After two days, all the animals appeared unkempt and apathetic, and after four days five mice were dead. Four further animals, which were moribund, were euthanized. The necropsy revealed peritonitis, ileus and a questionable bacterial infection that appeared to

originate in the intestinal tract. After 10 days, a further animal died. Repetitions of the experiment led to a mortality of 30-58% (Lieggi et al. 2005).

Summary and comment

The properties and side effects of anaesthesia with Avertin®, which have been known for decades from its use in humans, have now also been observed and sufficiently documented in small rodents, especially in mice. In human and veterinary medicine, Avertin® is now obsolete, having been superseded a long time ago by modern anaesthetics that are substantially more advantageous. These are also available for mice, and some of these substances are particularly useful in mice. They can be used both for injection anaesthesia and for inhalation anaesthesia, the latter being preferable in view of their greater safety. Alternative anaesthesia protocols that have not shown any interference with the experimental objectives are published in particular for use in generating genetically modified mouse lines (Zeller et al. 1997, Rulicke 2004, etc.), transgenic rat lines (Smith et al. 2004) and also for echocardiography in mice (Chu et al. 2006).

The further use of TBE should be confined to the continuation of ongoing studies in which evidence shows that a change in the method of anaesthesia influences the results of experiments.

In these cases, because of the risks involved in the use of TBE, utmost caution, reliability, expertise and experience are required in the preparation and handling of the injection solution. If these requirements cannot be met, a modern, standardized method of anaesthesia must be applied.

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